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INHIBITION OF N-NITROSAMINE FORMATION IN BACON BY α-TOCOPHEROL-COATED SALT SYSTEMS

By

Sreeram K. Reddy

A DISSERTATION

Submitted to

Michigan State University

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ABSTRACT

INHIBITION OF N-NITROSAMINE FORMATION IN BACON
BY α-TOCOPHEROL-COATED SALT SYSTEMS

By

Sreeram K. Reddy

The effects of α-tocopherol-coated salt systems on the inhibition of N-nitrosamine formation were investigated in both dry cured and brine cured bacon. In dry cured bacon, approximately a 96% reduction in N-nitrosopyrrolidine (NPYR) levels in the fried bacon was achieved with an ingoing α-tocopherol level of 500 mg/kg. NPYR levels in the cook-out fat were reduced approximately 92% using the same level of α-tocopherol. Bacon prepared by a small scale commercial operation, also contained reduced levels of NPYR when processed with α-tocopherol-coated salt, despite the high levels (>100 mg/kg) of residual sodium nitrite in the finished bacon. α-Tocopherol also appeared to be more effective as a blocking agent (91% versus 73% inhibition of NPYR) compared to the mixed tocopherols.

Brine cured bacon was also manufactured using brines containing α-tocopherol-coated salts. In order to achieve greater stability in the brine system, lecithin was also coated on the surface of the salt. Results of the N-nitrosamine analyses indicate a very high degree of inhibition of NPYR formation in both fried bacon (approximately 90%)

and the cook-out fat (97-100%) with an ingoing α-tocopherol level of 500 mg/kg. α-Tocopherol was also effective in minimizing N-nitrosodimethylamine (NDMA) formation in fried bacon. However, there was a wider variation (76-92%) in the percent inhibition obtained for NDMA. Less inhibition of NDMA formation in the cook-out fat was also observed.

The possible relationship of unsaturated fatty acids to the formation of N-nitrosamines in bacon was evaluated by swine feeding trials in which pigs were fed regular, α-tocopherol-enriched and corn oil-supplemented diets. Fatty acid analyses of the bacon indicated an approximate two fold increase in linoleic acid in bacon made from pigs fed the corn oil-supplemented diet. No obvious effects on the formation of NPYR were observed in bacon manufactured from the pigs. However, bacon made from swine fed the corn oil-supplemented diets contained much higher levels of NDMA (an approximate six-fold increase) than the control bacon. These results may indicate that the high levels of unsaturated fatty acids in bacon adipose tissue may enhance the formation of NDMA in fried bacon.

As a token of my appreciation
this dissertation is dedicated to
my late father Sri. K.V. Narayan Reddy
and mother Satya Devi

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INTRODUCTION

N-Nitrosamines, formed principally from the reaction of naturally occurring secondary amines with nitrites that may be added to foods or produced by bacterial reduction of nitrates, have been identified in many food systems including cured meat products, non-fat dried milk, dried malt and beer (Gray, 1981). The majority of these compounds have been tested in animal experiments and shown to be carcinogenic, mutagenic, embryopathic, or teratogenic. The presence of N-nitrosopyrrolidine (NPYR), and to a lesser extent, N-nitrosodimethylamine (NDMA) in fried bacon, cook-out fat and the vapors produced during the frying process has resulted in many investigations into the mode of formation of these compounds (Gray and Randall, 1979). Similarily, much effort has gone into developing means of supressing N-nitrosamine formation in fried bacon. Bharucha et al. (1979) have suggested that NPYR formation during frying of bacon occurs essentially, if not entirely, in the fat phase after the bulk of the water is removed, and therefore by a free radical rather than an ionic mechanism. They postulated that the essential but probably not the only requirements for a potential N-nitrosamine blocking agent in bacon are its (a) ability to trap NO. radicals, (b) lipophilicity, (c) non-steam volatility, and (d) heat stability up to 174^{o}C (maximum frying temperature). These appear important requirements since the precursors of NPYR have been associated with bacon adipose tissue (Fiddler et al., 1974).

Consequently, several lipid-soluble compounds have been examined as inhibitors of N-nitrosamine formation in bacon including ascorbyl palmitate (Sen et al., 1976), long chain acetals of ascorbic acid (Bharucha et al., 1980) and α-tocopherol (Fiddler et al., 1978). The latter compound, when dispersed with Polysorbate emulsifiers to obtain adequate distribution in the product, has been reported to reduce N-nitrosamine levels significantly in bacon when used at a concentration of 250-500 mg/kg. It has also been demonstrated that α-tocopherol disperses quite effectively during frying of bacon slices; therefore, application to bacon may be made by spray or dip to overcome the problem of water insolubility (Mergens and Newmark, 1979). Recently, Hoffmann-LaRoche, Inc. has filed a petition proposing affirmation that dl-α-tocopherol is generally recognized as safe for use in blocking N-nitrosamine formation in bacon (Federal Register, 1980).

To date, the majority of the bacon studies have centered on the formation and inhibition of N-nitrosamines in brine-cured bacon. Two recent investigations, however, have indicated the presence of high concentrations of NPYR in dry cured bacon after frying. NPYR levels ranging from 39-89 μg/kg were reported by Pensabene et al. (1979) while those cited by the Nitrite Safety Council (1980) ranged from traces to 320 μg/kg. These findings have identified dry cured bacon as one cured meat product category requiring further study. Evaluations of cure formulation changes or process control adjustments which may reduce or eliminate N-nitrosamine formation in dry cured bacon are necessary (Nitrite Safety Council, 1980).

The present study was undertaken to develop alternative means of suppressing N-nitrosamine formation in fried bacon. Specific objectives of the present study were: (1) to investigate the potential of α-tocopherol-coated salt systems, as N-nitrosamine blocking agents in fried dry cured bacon; (2) to investigate the commercial applicability of α-tocopherol-coated salt systems in inhibiting N-nitrosamine formation in dry cured bacon; (3) to study the effectiveness of α-tocopherol- versus mixed tocopherols (a mixture of α, β, γ and δ) - coated salt systems in inhibiting N-nitrosamine formation in dry cured bacon; and (4) to estimate the inhibition of N-nitrosamine formation in bacon pumped with a curing brine containing α-tocopherol and lecithin-coated-salt systems.

In another phase of this investigation, the free radical nature of the mechanism of N-nitrosamine formation was evaluated by swine feeding trials in which pigs were fed regular, α-tocopherol-enriched and highly unsaturated fat diets.

LITERATURE REVIEW

Chemistry of Formation

N-Nitroso Compounds

During the last ten years the occurrence of N-nitroso compounds has been demonstrated in foods by investigations in several countries. The majority of the N-nitroso compounds that have been tested in animal experiments have shown carcinogenic, mutagenic, embryopathic and teratogenic properties (Preussmann et al., 1976; Gray and Randall, 1979; Wishnok, 1979). Thus it is understandable that the possible occurrence of these compounds in food has caused considerable concern.

Traditionally, human exposure to N-nitroso compounds was confined to those associated with cured meat products. However, it has since become apparent that N-nitroso compounds are ubiquitous in the environment, particularly in many chemical, agricultural and consumer products (Fine et al., 1977). They also can be readily formed under the physiological conditions of the stomach and intestines, from ingested amines and nitrite, thus adding another dimension to the problem (Sander, 1967; Sen et al., 1969; Lijinsky et al., 1970). However, nitrite-preserved foods, especially bacon, have continued to be center of many research activities.

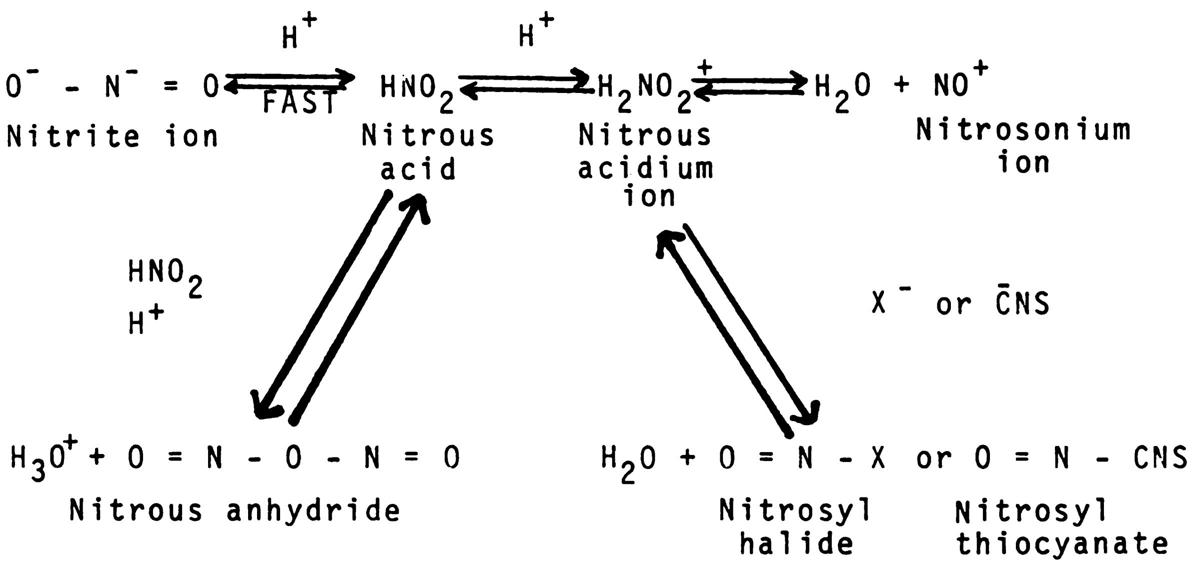
The formation and occurrence of N-nitrosamines and their toxicological and human health effects have been adequately documented (Scanlan, 1975; Crosby and Sawyer, 1976; Gray and Randall, 1979; Sen, 1980). N-Nitroso compounds can be divided into two groups according to their chemical reactivity: (1) N-nitrosamines, 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.

Kinetics of N-nitrosation Reactions

N-Nitrosamines are formed principally from the reaction between secondary amines and nitrous acid (Mirvish, 1975). N-Nitrosamines can also be formed, although to a lesser extent, from tertiary amines, quaternary ammonium compounds and primary polyamines (Gray and Randall, 1979). In addition, the formation of relatively non-volatile N-nitroso compounds have been suggested by model system studies (Mirvish, 1971; Kakuda and Gray, 1980).

Mirvish (1975) stated, that for nitrosation to occur, nitrite must first be converted to nitrous acid (HNO_2 - pK_a 3.36), indicating that the reaction is catalyzed by acid. Nitrous acid is then converted to 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^+) and nitrosyl halide (NOX).

The following reactions have been reported by Mirvish (1970) and show the equilibrium equations for the nitrite ion in aqueous solution:



The kinetics of N-nitrosamine formation from secondary amines and nitrous acid has been studied in detail by Mirvish (1972; 1975). Mirvish (1975) reported that the nitrosation reaction for most secondary amines proceeds via the active nitrosating species, nitrous anhydride. He further 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 equation shows that the reaction rate is proportional to the concentration of the free amine and to the square of the concentration of nitrite. Mirvish (1975) stated that since it is the free amine and not the protonated amine that is nitrosated, both pH and amine basicity may influence N-nitrosamine formation. Sander et al. (1972) found that there is an inverse relationship between the basicity of amines and the ease of nitrosation. In other words, the lower the basicity of a secondary amine, the easier it is to achieve nitrosation in acid solution. Sander et al. (1972) pointed out that at increasing acid concentrations, a higher proportion of the nitrite is converted to nitrous anhydride, which is the nitrosating agent. At the same time, however, salt formation by the amines is enhanced. Thus, there is an optimal pH value for the nitrosation of secondary amines which is about pH 3.

Precursors of N-Nitrosamines in Food Systems

Nitrates, Nitrites and Oxides of Nitrogen

The major sources of nitrate and nitrite intake are food and water. Nitrate is present virtually in all biological materials and constitute the primary source of fixed nitrogen in green plants. It occurs in high concentrations (sometimes as high as 1,000 - 3,000 mg/kg) in vegetables such as cabbage, cauliflower, carrots, celery, lettuce, raddish, beets, spinach and others (Ashton, 1970; White, 1975; Aworth et al., 1978; Lin and Lue, 1979; Lin and Yen, 1980). Nitrate also occurs in water, especially in well water, in some rural areas (Comly, 1945; Burden, 1961), fruits and fruit juices, baked goods, cereals, milk and other dairy products (Food and Drug Administration, 1980; U.S. Department of Agriculture, 1980; White, 1975, 1976). The concentration of nitrite in vegetables and water, 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, resulting from the reduction of nitrate by a variety of microorganisms that inhabit the mouth. They reported that the level of the salivary nitrite 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. These chemicals are used in many countries for the preservation of fish, meat, cheese and other food products. They are mainly used for their role in inhibiting the outgrowth of Clostridium botulinum spores and retard possible botulinal toxin development (Christiansen et al., 1973; Tompkin et al., 1978; Lucke and Leistner, 1979). Nitrite also 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). It also provides a potent antioxidant effect and helps to prevent the development of warmed-over flavor (Bailey and Swain, 1973; Fooladi et al., 1979; Pearson et al., 1977; MacDonald et al., 1980).

Varying concentrations of nitrogen oxides are found in air outdoors, indoors, in the workplace, tobacco smoke, and during processing of foods such as smoking (Ehrenberg et al., 1980; Eslandsson, 1981; Newmark and Mergens, 1981b; World Health Organization, 1978). Nitrogen oxides 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 (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. The formation of N-nitroso compounds from oxides of nitrogen is usually faster and more extensive than from aqueous nitrous acid (Challis and Kyrtopoulos, 1979). The average concentration of nitrogen oxides in the atmosphere is approximately 90 $\mu g/m^3$, although their concentration in air of smog laden cities may reach 1 mg/kg ($\sim$1,888 $\mu g/m^3$).

In domestic kitchens, peak levels of nitrogen dioxide may also reach concentrations of 1 mg/kg (Moschandreas et al., 1977). Estimates of the daily intake of nitrate and nitrite from this source range widely. Erlandonson (1981) estimated that the average exposure for Gothenberg, Sweden of 1-2 mg of nitrate and 0.9 mg of nitrite would result from an average concentration of 114 $\mu g/m^3$. In the United States, Newmark and Mergens (1981a) observed 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. A summary of the various figures used for average concentration of nitrate, nitrite and nitrogen oxides are given in Table 1.

White (1975, 1976), Food and Drug Administration (1979), 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. The data from these studies show that vegetables are the major source (86-97%) of nitrate in the average American diet; the remaining nitrate originates from salivary excretion and cured meats (0-9%). The predominant portion of the ingested nitrite, however, comes from the bacterial reduction of nitrate in the saliva (76.8%) and a significant amount from cured meats (61-91%).

Table 1. Average concentrations of nitrate, nitrite and nitrogen oxides used to estimate exposure of humans[a].

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).

Various studies have shown that the nitrite level in saliva can increase markedly after consumption of nitrate-rich foods such as vegetables (Spiegelhalder et al., 1976; Tannenbaum et al., 1976). These results imply that the ingested nitrate is converted in vivo in the human body to nitrite and then excreted in the saliva. Since the volume of daily excretion 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 important in the formation of N-nitrosamines in the human stomach. According to the results obtained by Tannenbaum (1978) and Tannenbaum et al. (1978), nitrite can apparently also be produced in the upper and lower gastrointestinal tract, thus complicating the situation further. However, Witters et al. (1979) questioned the validity of the findings of Tannenbaum et al. (1978) and concluded that the nitrite found in the ilium, urine and faeces could be due to the depletion of body stores of nitrite and nitrate and the passage of nitrate down the gut.

Although the intake of nitrogen oxides contributes to the daily exposure to nitrite and nitrate, the concentration from this source is relatively small for the average U.S. population (National Academy of Sciences, 1981). However, peak levels of nitrogen oxides in smoked foods, tobacco smoke, smog laiden cities can result in substantial exposure to both nitrate and nitrite.

Amines in Foods

Amines and amino compounds are a diverse group of chemicals whose reactivity with nitrosating agents varies considerably, especially in their respective environmental media (e.g., air, food, drugs, etc). As discussed earlier, most secondary amines, N-alkylureas and N-alkylcarbamates 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.

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 histidine (Dierick et al., 1974); (b) trimethylamine oxide to trimethylamine (Tarr, 1940); (c) aldehyde amination as in the amination and transamination of aldehydes, which is the potential pathway for 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 amino 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). Valisek and Davidek (1974) also postulated that amines in foods could easily be formed during the nonenzymatic browning process.

A number of volatile amines including methylamine, dimethylamine, trimethylamine, ethylamine, n-propylamine and isopropylamine have been reported in uncured pork belly by Patterson and Mottram (1974). These authors also reported that the concentrations of dimethylamine, trimethylamine, n-propylamine, and isopropylamine increased during the manufacture of bacon. Of these, dimethyl- and trimethylamine contribute to the formation of NDMA in cured pork meats (Patterson and Edwards, 1975; Gray et al., 1978). Rice et al. (1976) have reported the presence of histamine, putrescine, tyramine, 2-phenylethylamine in dry and semi-dry sausages. Various amines (dimethylamine, di-n-propylamine, pyrrolidine, morpholine and piperidine) have been detected at levels of 2 μg/kg or less in baked ham (Singer and Lijinsky, 1976).

Low levels of simple amines have also been reported occasionally in various meat products (Landmann and Batzer, 1966; Cantoni et al., 1969; Patterson and Mottram, 1974). The monoamines (histamine, tryptamine, tyramine, and ethanolamine) and polyamines (spermine, spermidine, putrescine and cadaverine) have been identified in fresh pork bellies (Spinelli et al., 1974) at concentrations ranging from 0.03 mg of cadaverine to 8.0 mg for spermine per 100 g tissue. Processing into bacon did not significantly alter the amine content. Similar amines were identified in fresh hams 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 may be due to volatilization, while significant increases in spermine, spermidine, putrescine and cadaverine occurred during putrefaction.

Among the amino compounds that have been quantitated in pork bellies are the free amino acids proline, alanine, isoleucine, leucine, methionine, phenylalanine, tyrosine, valine, glutamic acid, cysteine, and aspartic acid as well as hydroxyproline and sarcosine (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.

Recently, Mirvish and Cairnes (1981) reported that creatinine was the major precursor to methylurea formed in a Japanese dried bonito fish. In this study, nitrosation-denitrosation of creatinine produced 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). 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). A dried fish product and fried bacon were found to contain 2 - 3 g/kg creatinine (Mirvish and Cairnes, 1981). Since these nitrosation reactions occurred with a very large molar excess of sodium nitrite, it is doubtful whether these compounds could be formed in cured meat products.

Fairly high levels of dimethylamine, trimethylamine and trimethylamine oxide have been detected in various fish, particularly those of marine origin (Shewan, 1951; Castell et al., 1971; Golovnya, 1976). Similar amines at mg/kg levels were reported in mature Gouda cheese (Ruiter, 1973). In addition, a wide range of simple aliphatic and monoamines such as tyramine, histamine, and tryptamine has 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 concluded that modified powdered milk contains about five times as much dimethylamine as milk, while the amounts in butter and cheese were trace.

Polyamines such as cadaverine, putrescine, spermidine and spermine are found in the germs of cereals such as barley, rice, oats, corn, wheat and sorghum (Moruzzi and

Caldarera, 1964). Surprisingly, paprika, cayenne pepper and black pepper were found to contain fairly high levels of cyclic amines, pyrrolidine and piperidine (Marion, 1950; Gough and Goodhead, 1975). Since spices are used in the preparation of various foods in different countries, they may contribute significantly to the total intake of amines in our diet.

In light of the above findings on the precursors of N-nitrosamines, the presence of amines and nitrate, nitrite or oxides of nitrogen in human diets is therefore, unavoidable, even without the consumption of cured meat items.

N-Nitrosamines in Cured Meat Systems

Extensive compilations of the volatile N-nitrosamine content of foodstuffs in various diets including wheat flour, mushrooms, alcoholic beverages, cheese, milk and soybean oil as well as in meat and fish products have been published 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 item of major importance as far as the formation of N-nitrosamines are concerned are the cured meat products. The occurrence of N-nitrosamines in cured meats have been summarized by Sen (1980) and Walters (1980). It should be pointed out that except in a few cases, the levels of N-nitrosamines detected were extremely low and even these were detected only in a small percentages of the samples tested. One very important consideration is that cooked bacon, nitrate- or nitrite-treated smoked fish and certain types of salted and dried fish are the main contributors of N-nitrosamines in our diet. The major N-nitrosamines detected in these foods are N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR) and N-nitrosopiperidine (NPIP).

Cured Meats Other Than Bacon

A considerable range of cured meat products other than bacon has been examined for the presence of volatile N-nitrosamines. For instance, the early work of several researchers (Sen, 1972; Wasserman et al., 1972; Panalaks et al., 1973) indicated that fairly high levels (sometimes as high as 25,000 μg/kg) of NDMA, NPYR and NPIP were sporadically found in corned beef, luncheon meat, smoked beef, frankfurters, pork, continental sausages and salami. Both the levels and frequency of occurrence of these N-nitrosamines were very unpredictable. The reason for this inconsistency was not clear until later when Sen et al. (1973a; 1974) related the 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). Both black pepper (piper nigrum) and paprika (*capsicum annum*) can react with nitrite to form both NPIP and NPYR, respectively. This then led to changes in Canadian and United States regulations requiring that the curing agents and spices be packaged separately. This action has resulted in marked decreases in the levels of N-nitrosamines in various cured meat products (Sen and McKinley, 1974; Sen et al., 1976b; Eisenbrand et al., 1977; Gough et al., 1977).

More recently, the presence of N-nitrosamines in cured meats other than bacon has been the subject of several surveys (Sen et al., 1979; Nitrite Safety Council, 1980; Holland et al., 1982). In general, the majority of the positive samples contained extremely low levels of N-nitrosamine, usually less than 1 μg/kg (Sen et al., 1979; Nitrite Safety Council, 1980). In the Holland et al. (1982) study, the predominant N-nitrosamines detected were NDMA and N-nitrosomorpholine (NMOR) and, generally, values of 4 μg/kg were obtained for each N-nitrosamine. In a similar study, Gray et al. (1981) detected the formation of NMOR at a level of not more than 4 μg/kg except for two samples which contained 8 and 11 μg/kg in heated chicken frankfurters which have been prepared with various levels of nitrite (0 - 156 mg/kg). It has been suggested that the detectable levels of NMOR in this study and the Canadian (Holland et al., 1981) study were attributed 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 almost invariably after cooking, the levels depending on cooking conditions and other less well defined factors (Pensabene et al., 1980). Interestingly, the amounts of N-nitrosamine detected in the cooked bacon or rendered fat constitute only a portion of the total quantity of N-nitrosamine formed. During frying, a substantial portion of these compounds is volatilized in the fumes. This phenomenon has been investigated by several workers who reported a wide range of values for the percentages of N-nitrosamines found in the vapor (Table 3). Obviously the mode of cooking, as well as the moisture content and ratio of lean to adipose tissue in the bacon samples influence the amount of N-nitrosamines in the vapor.

Recent data on NPYR in cooked bacon samples (Greenberg, 1976; Sen et al., 1977; Havery et al., 1977 and 1978) indicate progress in decreasing the levels of NPYR in fried bacon. 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 N-nitrosation inhibitor, ascorbate, in the bacon curing mixture (Havery et al.,

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. (1979a)	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-nitrosamine in the fumes 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
Warthesen et al. (1976)	20-40	-	pork belly[b]
Sen et al. (1976c)	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 32	Up to 62	bacon

[a]Adapted from Gray (1981).

[b]Contained added nitrite.

1978). The amounts of NPYR formed in cooked bacon is also influenced by the method of cooking, frying temperature, cooking time (Gray, 1981), slice thickness (Theiler et al., 1981a), curing solution ingredients (Theiler et al., 1981b), and belly composition (Amundson et al., 1982a) and handling (Amundson et al., 1982b).

Mechanism of NPYR Formation

The consistent occurrence of NPYR in fried bacon and cook-out fat has led to an intensive search for both the precursors and mechanism that could account for its formation. Although model system studies have implicated a number of compounds including proline, collagen, putrescine, spermidine, pyrrolidine (PYR) and glycyl-L-glycine as possible precursors of NPYR, the most probable precursor of NPYR in bacon appears to be proline. 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).

How proline is converted to NPYR has not yet been fully elucidated and could conceivably occur by either of two pathways (Gray, 1976; Bharucha et al., 1979) (Figure 1). One pathway involves the initial N-nitrosation of proline, followed by decarboxylation, while in the other, proline is first decarboxylated to PYR followed by N-nitrosation to NPYR. Since the conversion of N-nitrosoproline to NPYR

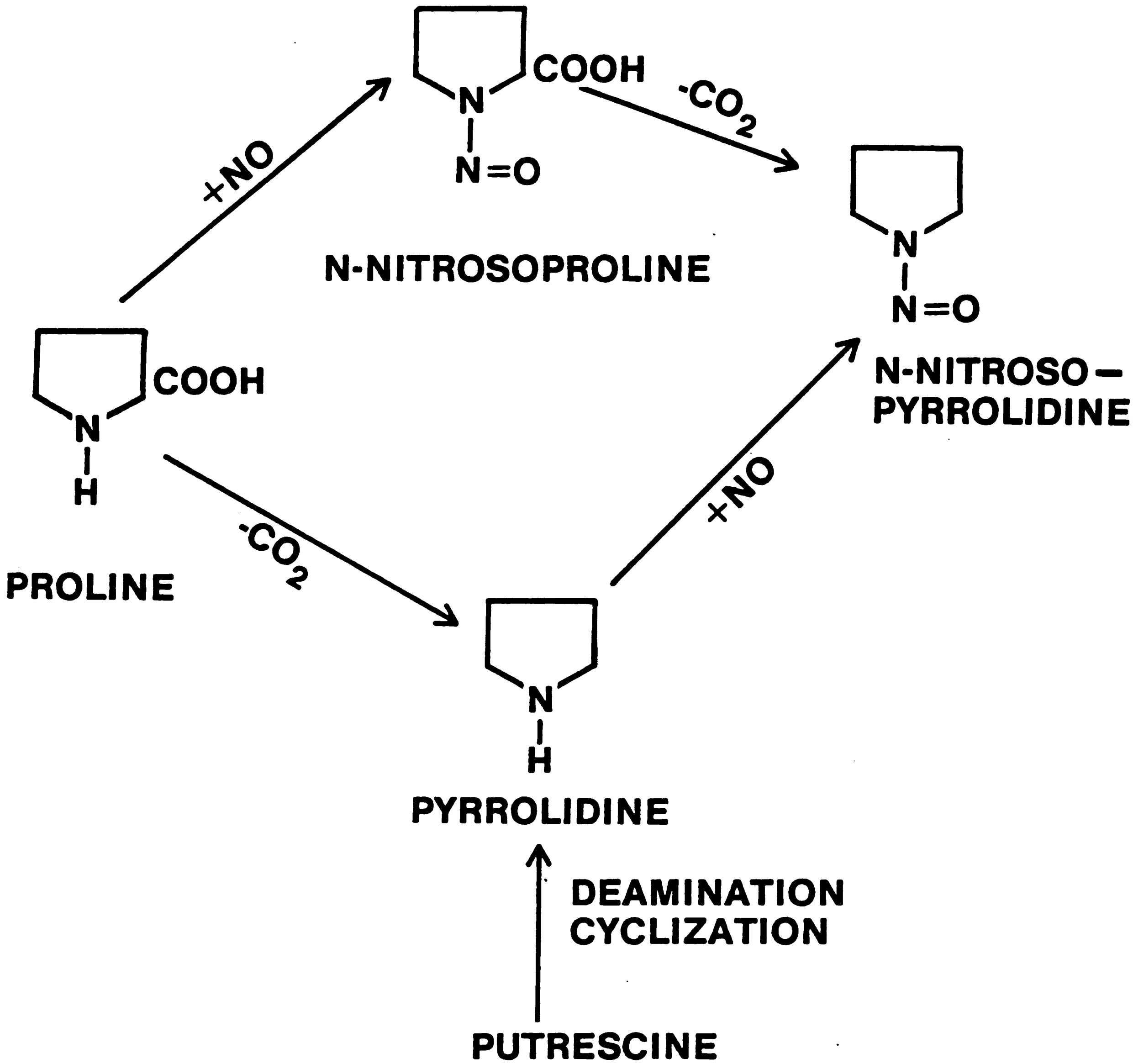


Figure 1. Possible pathways of N-nitrosopyrrolidine formation in bacon (Gray, 1976).

occurs at a much lower temperature than the transformation of proline to PYR, the pathway involving the intermediacy of N-nitrosoproline is thus the more likely route (Bharucha et al., 1979). It has been reported that preformed N-nitrosoproline in raw bacon is not the primary precursor of NPYR in cooked bacon (Sen et al., 1976a; Hansen et al., 1977; Bharucha et al., 1979), as shown by the fact that ascorbyl palmitate, when added to bacon, inhibits the formation of NPYR (Sen et al., 1976a). However, this by no means rules out the intermediacy of N-nitrosoproline which could be formed at the higher temperatures attained during the frying process (Bharucha et al., 1979). The evidence presented by Lee (1981) also strongly suggests that the major pathway of NPYR formation in fried bacon is via the N-nitrosation of proline followed by the decarboxylation of N-nitrosoproline, the yield limiting step being the decarboxylation reaction.

The mechanism of NPYR formation has been studied by Coleman (1978) who reported that the requirement of a high temperature, the inhibitory effects of water and antioxidants, and the catalytic effects of a lipid hydroperoxide 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 toward the end of the frying process, N-nitrosamine formation during frying of bacon 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 speculated that, during the frying of bacon, nitrous acid is converted essentially into N_2O_3 by continuous removal of water, and N_2O_3, in turn, undergoes dissociation at higher temperatures (>>100°C) to NO· and $NO_2^{\cdot}$ (Figure 2). Since NO· is relatively stable, it was

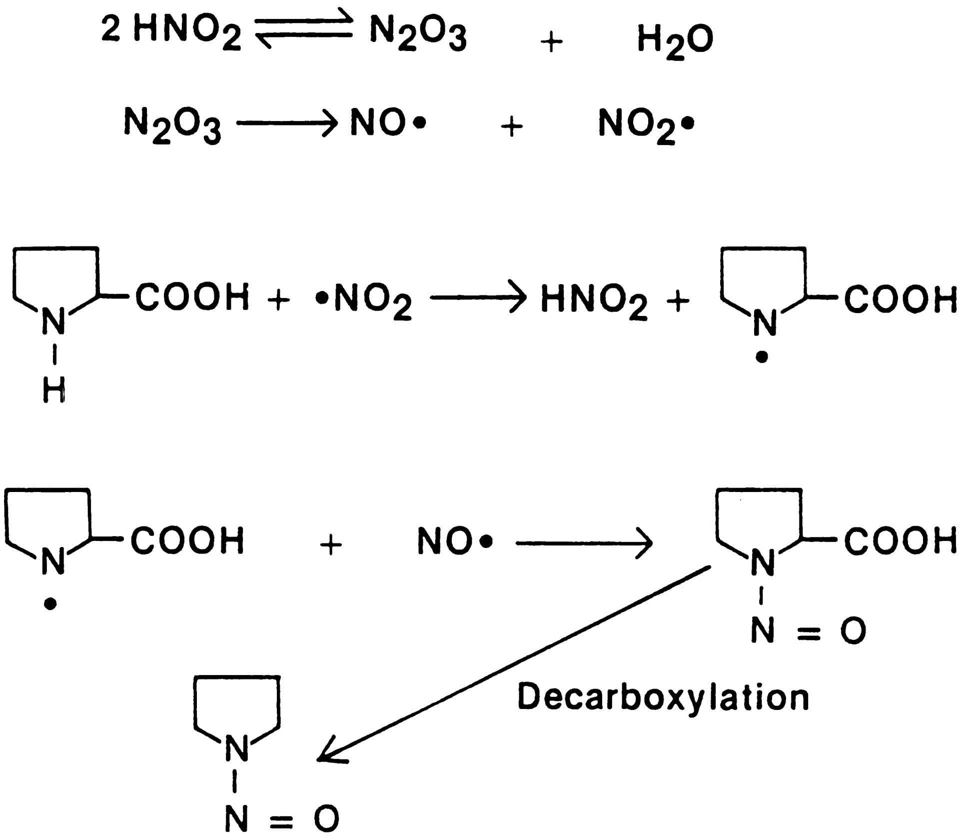


Figure 2. Free radical mechanism of N-nitrosopyrrolidine formation in bacon (Bharucha et al., 1979).

concluded that the $NO_2^{\cdot}$ radical can act as the chain initiator and abstract the amino proton from PRO to give a radical which combines with the NO$^{\cdot}$ radical to give NPRO as shown (Bharucha et al., 1979).

Mechanism of NDMA Formation

While most of the recent N-nitrosamine research has centered on NPYR and its precursors in bacon, information on formation and precursors of other volatile N-nitrosamines is still lacking (Gray and Randall, 1979). Although NDMA has been reported in fried bacon, there have been very few reports as to the actual precursors and mechanism of formation of this N-nitrosamine. 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 (Eisenbrand et al., 1976; Ender and Ceh, 1971) and lecithin (Mohler and Hallmayer, 1972; Pensabene et al., 1975). Gray et al. (1978) investigated various compounds, all of which were endogenous to bacon as possible precursors of NDMA in bacon. Under conditions normally encountered in the pan frying of bacon, choline-containing compounds and sarcosine produced measurable quantities of this N-nitrosamine. Patterson and Mottram (1974) quantitated the volatile amines in the loin eye muscle of 10 pork carcasses at various stages of curing. Values of DMA below 200 μg/kg before curing were reported while up to 520 μg/kg of DMA were found in vacuum stored bacon. There have been little evidence on the sarcosine content of pork belly.

Factors Influencing NPYR Formation

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

Cooking methods. It has been well established that pan frying of bacon results in more NPYR formation than other cooking procedures such as microwave cooking (Herring, 1973; Pensabene et al., 1974) and grilling (Bharuch et al., 1979). Bharucha et al. (1979) explained the reduced yields of N-nitrosamines during grilling as being due to the cook-out fat running out of heated area. Consequently, the bacon slices never reach the same temperature as during pan frying. It has also been demonstrated that both frying temperature and time clearly influence the levels of NPYR in cooked bacon. Pensabene et al. (1974) showed that bacon samples from one belly formed no NPYR when fried for 105 min at 99^{o}C, while samples from the same belly, fried to the same degree of "doneness" at 204^{o}C for 4 min, produced 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 min at 360°C, after starting with a cold frying pan. Very little N-nitrosamine was found in the rendered fat after 4 min of heating; however, the N-nitrosamine level increased sharply with time and reached a maximum at around 12 min and then began to decline. Two explanations were offered for the initial low formation of N-nitrosamines; (a) the N-nitrosamines were actually formed at about 100°C, but being steam-volatile, were removed with the water vapor; or (b) the N-nitrosation 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 at length (Mirvish, 1970; Mirvish, 1975) and, 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. Therefore, it is not surprising that the amount of nitrite permitted in bacon has received considerable attention. Although there have been suggestions that it is the initial and not the residual nitrite that influences N-nitrosamine formation in bacon (Sen et al., 1974), recent evidence seems to indicate that the lowest residual nitrite gives the least probability of N-nitrosamines being formed (Dudley, 1979; Sebranek, 1979). Consequently, 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, cooking or fermentation (Gray, 1976).

Recently, Robach et al. (1980) investigated the effects of various concentrations of sodium nitrite and potassium sorbate on N-nitrosamine formation in commercially prepared bacon. 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. This marked reduction in NPYR levels is clearly due to the reduced levels of nitrite, although it has been reported that sorbic acid also possesses anti-N-nitrosamine activity (Tanaka et al., 1978).

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 ($p<0.05$) NPYR than that made from bellies that has 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 investigators (Lakritz et al., 1976; Gray and Collins, 1977b) have shown that the free proline content in whole and lean tissue of green pork bellies increased approximately 50% after storage at 2°C for 1 week. Over the same period, free proline in the adipose tissue increased approximately 90%.

Smoking. The effects of smoking on the formation of N-nitrosamines in bacon has been investigated recently by Bharucha et al. (1980). They reported that unsmoked bacon samples generally tended to contain more N-nitrosamines, presumably because of their higher nitrite content at the time of frying. Sink and Hsu (1977) showed a lowering of residual nitrite in a liquid smoke dip process for frankfurters when the pH also was lowered. The effects of smoke seems to be a combination of pH decrease and direct C-nitrosation of phenolic compounds to lower the residual nitrite in the product (Knowles, 1974). This is an area which requires further study since certain C-nitrosophenols have been shown to catalytically transnitrosate amines in model systems (Davies et al., 1980).

N-Nitrosamine Blocking Agents or Inhibitors

Model Systems

The formation of N-nitroso compounds can be reduced, minimized or even completely prevented by the presence of blocking agents when a nitrosation potential exists. According to Mergens and Newmark (1980), a blocking agent is essentially a substance capable of rapidly reducing the nitrosating agent to the non-nitrosating nitric oxide or oxides of nitrogen of lower oxidation state. 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).

Mirvish et al. (1972b) first discovered that the ascorbic acid was an effective inhibitor of the nitrosation reaction in aqueous systems. Since then, a large number of 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, glutathione, methionine), urea, as well as ascorbic acid and its derivatives. The efficiency of these compounds as 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 an excellent blocking agent against nitrosation, particularly in weakly acidic conditions (Mirvish et al., 1972b). The chemical reaction is nitrite reduction to nitric oxide (NO), linked with ascorbic acid oxidation to dehydroascorbic acid (Figure 3).

Since the active form of nitrous acid for N-nitrosamine formation is N_2O_3 (Mirvish, 1981), the ascorbic acid directly competes with amines for the same nitrosating species. The properties and utility of ascorbic acid as a blocking agent have been very well described in the literature (Kamm et al., 1973, 1975, 1977; Gray and Dugan, 1975; Archer et al., 1975; Mirvish, 1977; Mergens et al., 1978; Mirvish, 1981; Newmark and Mergens, 1981).

Ascorbic acid is not effective in blocking nitrosation reactions in two-phase model systems composed of aqueous and non-polar components. 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 two-phase model system containing sodium ascorbate. They believed 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.

Mergens and Newmark (1980) discussed the complex nature of food composition and its effects of N-nitrosamine formation. Lipids, when present, can act simultaneously as

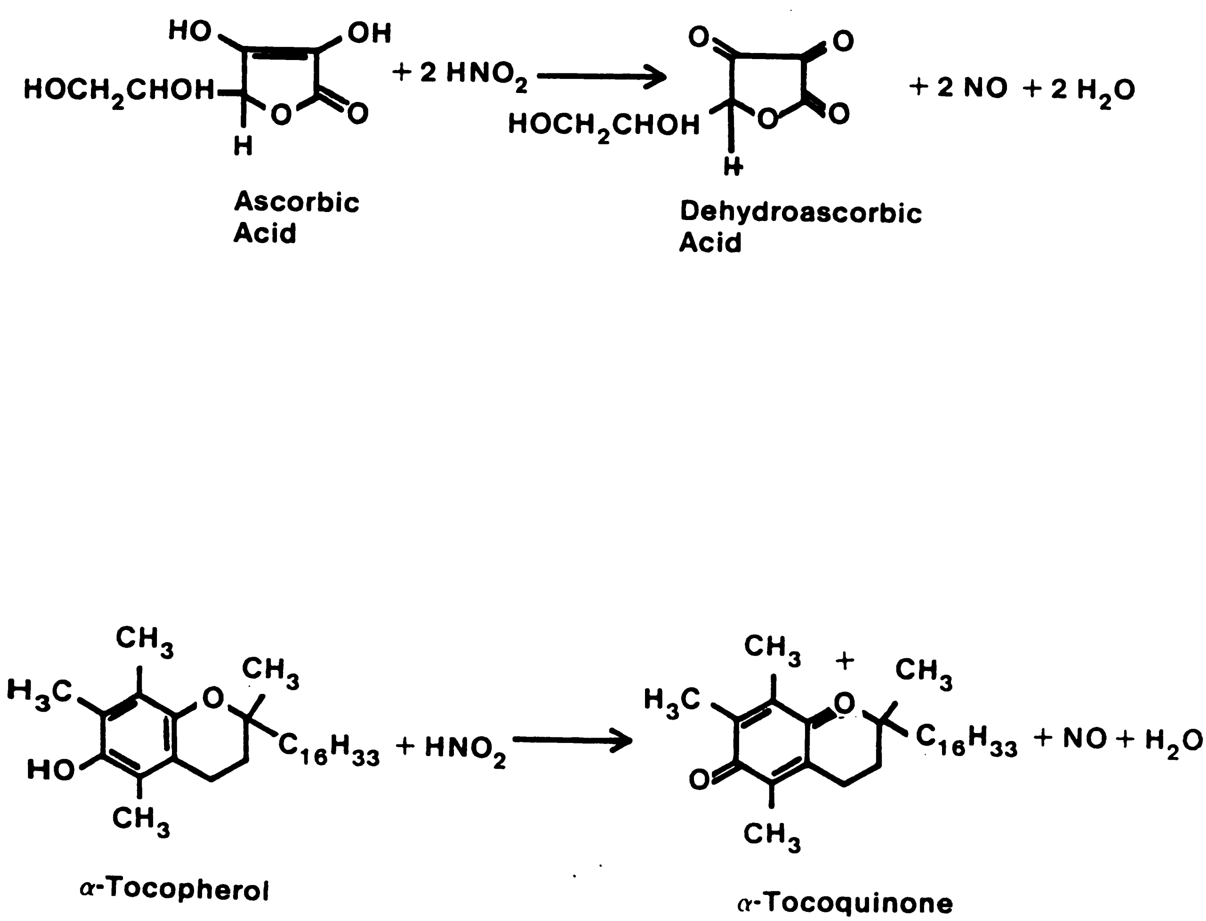


Figure 3. Reactions of nitrite with ascorbic acid and α-tocopherol.

a ready solvent for the unprotonated free base substrate and nitrous anhydride (N_2O_3), resulting in an extremely rapid nitrosation reaction. Removal of the water phase of a food, such as the case during the frying of bacon is a typical example. 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 nitrosation occurs. Consequently, α-tocopherol has been shown to be a very effective inhibitor of the nitrosation reaction (Pensabene et al., 1978).

The structure of α-tocopherol permits it to be particularly effective in inhibiting N-nitrosamine formation. It functions as a blocking agent because it has the ability to reduce nitrite, while α-tocopherol itself is oxidized to a quinone-type compound viz., α-tocoquinone (Figure 3). Since α-tocopherol does not have unsubstituted carbon atoms in the phenolic ring, it cannot form C-nitroso derivatives that might promote transnitrosation. On the other hand, β, γ and δ-tocopherols have unsubstituted positions in the phenol ring and are not as effective as α-tocopherol in inhibiting N-nitrosation, perhaps because they produce nitrosating C-nitroso compounds (Mirvish, 1981; Newmark and Mergens, 1981).

Although the majority of studies on blocking the nitrosation reaction has centered on the use of ascorbic acid or its derivatives and α-tocopherol, several other compounds or groups of compounds have been investigated. Polyphenolic compounds such as gallic acid as well as simple phenols can function as 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 shown that 1,2- and 1,4-dihydroxyphenols (including naturally occurring flavanols) inhibit N-nitrosamine formation at pH 4.0. On the other hand, 1,3-dihydroxyphenols (e.g. resorcinol) are powerful catalysts under similar conditions (Pignatelli et al., 1980).

Certain sulfur compounds can function as N-nitrosamine blocking agents. Bisulfite reduces nitrite in two steps (Hisatune, 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, but these compounds can themselves act as nitrosating agents in the absence of nitrite (Davies et al., 1978).

Recently, Newmark and Mergens (1981b) made an attempt to summarize their concepts of nitrosation reactions in aqueous solution, gas state, and nonpolar solvents (i.e. lipids), with the expected exchanges of nitrosating sources among the three phases. Only oxides or ions containing nitrogen in the +3 or +4 state can nitrosate. Oxidation to the +5 state (nitrate) removes the capacity to nitrosate, as does reduction to +2 state (nitric oxide, NO) or to the +1 state (nitrous oxide). Ascorbic acid in aqueous solution and α-tocopherol in lipid or nonpolar solvents or the lipid or micellar phase of an emulsion both act by reducing the nitrosating agent to NO. This study also suggests that in multiphase systems such as actually exist in tissues in vivo, in the contents of the GI tract, in many foods such as bacon, etc., both ascorbic acid and α-tocopherol should be used in combination to block nitrosation, in order to be more effective (Newmark and Mergens, 1981b). For example, since the active nitrosating intermediate N_2O_3 can rapidly move in and out of lipid and aqueous phases, blocking agents should ideally be present in both.

Bacon Systems

As hypothesized by Gray and Dugan (1975), any compound that can successfully compete with a meat mixture for reaction with nitrite is likely to lower the potential for N-nitrosamine formation. Consequently, several compounds have been investigated as potential blocking agents in bacon (Table 4). The first compound to be recognized as a N-nitrosamine inhibitor was ascorbic acid or its isomer,

Table 4. Effects of various blocking agents for N-nitrosamine formation in fried bacon.

Compound	Level mg/kg	Percent inhibition		Investigator
		Fried bacon	Cook-out fat	
Ascorbic acid	1000	100	-	Greenberg (1973)
Ascorbyl palmitate	1000	59-87	-	Sen et al. (1976a)
	500-1000	-	70-90	Bharucha et al. (1980)
Ascorbic acid acetal	1000	62-88	90-98	Bharucha et al. (1980)
Piperazine	1000	90-91	-	Sen et al. (1976a)
Propyl gallate	1000	50-97	-	Sen et al. (1976a)
TBHQ	500-1000	55-64		Anon (1977)
Alpha tocopherol	500	Up to 80	-	Fiddler et al. (1978)
	500	Up to 85		Mergens and Newmark (1979)

erythorbic acid (Mirvish et al., 1972a). This information has been utilized in regulatory form to ensure that all bacon is processed with maximum levels (550 mg/kg) of ascorbate or erythorbate (Sebranek, 1979). Although these compounds are quite effective, they are not completely successful as N-nitrosamine inhibitors because of their limited solubility in adipose tissue.

Consequently, ascorbyl palmitate has been found to be more effective than sodium ascorbate in reducing N-nitrosamine formation (Sen et al., 1976). Similar conclusions were reached by Bharucha et al. (1980), who showed that ascorbyl palmitate reduced N-nitrosamine formation in bacon by 70-90% when used at the 500-1000 mg/kg level. However, its activity tends to decrease with storage time. The long chain acetals (C_{12}, C_{14}, C_{16}, C_{18}, $C_{18:1}$) of ascorbic acid have also been reported to be effective in bringing about a 93-98% reduction of N-nitrosamines in the cook-out fat when streaked on bacon slices at a 1000 mg/kg level (Bharucha et al., 1980). The C_{12} ascorbyl acetal, and to a much lesser extent the C_{14} homologue left a soapy after-taste. However, the bacon samples treated with ascorbyl C_{16}, C_{18} and $C_{18:1}$ acetals were indistinguishable from commercial samples. The major drawback to this group of compounds as N-nitrosamine blocking agents in bacon is that they are not approved for use in food systems.

The inhibition of NPYR formation in fried bacon by the use of cure-solubilized α-tocopherol has been demonstrated by Fiddler et al. (1978). This compound was dispersed with Polysorbate emulsifiers to obtain adequate distribution in the product and resulted in a significant reduction of N-nitrosamines when used at a level of 250-500 mg/kg. Walters et al. (1976) also reported reduced levels of N-nitrosamines in the vapors during frying of bacon in fat containing α-tocopherol. More recently, it has been shown that α-tocopherol is dispersed quite effectively during the frying of bacon slices; therefore application to bacon may be made by spray or dip to overcome the problem of water insolubility (Mergens and Newmark, 1979).

Several other potential N-nitrosamine blocking agents that are lipid soluble have been studied. Sen et al. (1976) reported relatively successful use of propyl gallate when applied to the bacon slices immediately before frying. Tertiary butylhydroxyquinone (TBHQ) has also been reported to function as a blocking agent (Anonymous, 1977). In a recent study, Bailey (1980) prepared dry-salt cured bacon with various levels of nitrite. Unacceptable levels of NPYR were formed in bacon containing 120, 200, 400 and 625 mg/kg of sodium nitrite, but this was reduced to below 16 μg/kg for all samples except the 625 mg/kg sample by including 1 to 2% dextrose in the curing mixture.

Toxicology of N-Nitroso Compounds

Many N-nitrosamines and, in general, most N-nitroso-compounds are highly potent carcinogens. Thus far, about 130 N-nitroso compounds have been tested and approximately 80% of them shown to be carcinogenic (Preussmann et al., 1976). NDMA has been shown to be carcinogenic in 6 species and NDEA in about 20 animal species including some subhuman primates (Magee et al., 1976; Sen et al., 1980). Mink appear to be the most sensitive animal to the toxic and carcinogenic actions of N-nitrosamines (Koppang and Rime-slatten, 1976). NDMA, the simplest and the most widely occurring N-nitrosamine in foods, causes mostly liver tumors and occasionally kidney tumors, whereas methylbenzylnitrosa-mine and N-nitroso-n-butyl-(4 hydroxybutyl) amine causes cancer of the oesophagus and bladder, respectively (Druckrey et al., 1969; Magee et al., 1976). Hecker et al. (1979) studied the toxicity of cyclic N-nitrosamine, NPYR, which occurs most commonly in bacon. Their investigation confirmed the earlier studies (Preussmann et al., 1976; Cottrell et al., 1979) that NPYR produces hepatocellular tumors in rats. N-Nitrosamines are considered indirect acting car-cinogens and require metabolic activation (Lijinsky, 1977). N-Nitrodialkylamines (e.g. NDMA, NDEA) are highly potent, whereas N-nitrosamines with branching and consequently fewer hydrogens at the α-carbon (e.g. N-nitrosodiethanola-mine, N-nitrososarcosine) generally have lower carcinogenic potency. The α-position of N-nitrosamines has shown to be associated with the carcinogenic action of these compounds (Wishnok, 1979).

Carcinogenic N-nitroso compounds are also generally mutagenic, although the correlation of carcinogenicity and mutagenicity is not perfect. N-Nitrosamines require microsomal or host-mediated metabolism to become mutagenic, whereas the carcinogenic nitrosamides do not require metabolism for mutagenesis, i.e. they are direct-acting mutagens (Newmark and Mergens, 1981b).

EXPERIMENTAL

Bacon Manufacturing

Dry Cured Bacon

Pilot plant study. Dry cured bacon was manufactured under carefully controlled processing conditions in the pilot plant at the Meat Laboratory, Michigan State University. Pork bellies (approximately 10-12 pounds) were purchased from a local supplier soon after slaughter and stored for no more than 2 days in a cooler at 2^{o}C. The bellies were processed according to the treatments described in Table 5, six randomly selected bellies being used per treatment. The dry curing mixtures contained regular Alberger^{R} Fine Flake salt without added α-tocopherol (Diamond Crystal Salt Co., St. Clair, MI) for treatment 1, 2% α-tocopherol-coated Alberger Fine Flake salt for treatment 2 and 3, and 5% α-tocopherol-coated Fine Flake salt for treatment 4. The 2% α-tocopherol-coated salt was blended with regular fine flake salt in treatment 2 in order to achieve the correct ingoing level of α-tocopherol. The dl-α-tocopherol (Hoffmann-LaRoche Inc., Nutley, NJ) -coated salt systems were prepared by the Diamond Crystal Salt Company.

Table 5. Target or ingoing concentrations of dry curing ingredients (pilot plant study).

Treatment	Salt (%)	Sugar (%)	Sodium nitrite (mg/kg)	Sodium ascorbate (mg/kg)	α-tocopherol (mg/kg)
1	2.5	0.83	120	550	0
2	2.5	0.83	120	550	250
3	2.5	0.83	120	550	500
4	2.5	0.83	120	550	750

The dry curing ingredients were thoroughly mixed by hand before applying to the bellies. The rubbed bellies were placed in plastic bags (3 bellies per bag) and held in the curing room at 2^{o}C. The bellies were inverted after 5 days to allow for good distribution of the cure throughout the bellies. At the end of the curing period, the bellies were transferred to a laboratory smokehouse (Drying Systems, Inc., Chicago, IL) and smoke-cooked at a temperature of 58^{o}C (dry bulb) for 4 h followed by three further hours of smoking at 52^{o}C (dry bulb) and ambient relative humidity. Smoke was applied throughout cooking using a midget size Mepaco smoke generator (Meat Packers Equipment Co., Oakland, CA), utilizing hickory saw-dust. The smoked bellies were transferred to a tempering cooler (-2^{o}C) where they were held overnight prior to slicing and packaging.

The six bellies per treatment were sliced to 20-25 slices/lb or 1/11" thickness and packaged in a manner similar to that described by Robach et al. (1980). After each belly was sliced, it was packaged in 20 packages with the first slice going in to first package, the second slice into the second package, the third slice into the third package and so on until the belly was completely packaged. Thus, each of the 20 packages could be considered representative of an entire belly. Six packages randomly selected from each belly were analyzed for N-nitrosamines, while the remaining packages were used for nitrite, salt, α-tocopherol, moisture, fat, protein determinations and _Clostridium botulinum_ studies.

Small scale commercial study. In order to investigate the practical application of α-tocopherol-coated salt as an N-nitrosamine inhibitor, dry cured bacon was prepared by a local small scale commercial processor. This operation involved a 24 h dry cure followed by a 9 day immersion of the bellies in a brine cover pickle containing 1200 mg of nitrite/liter. Target levels of the dry rub were similar to those of treatment 3 (Table 5), i.e., 120 mg/kg of nitrite, 500 mg/kg of α-tocopherol and 550 mg/kg of sodium ascorbate. The α-tocopherol was introduced into the bellies by application of a 2% α-tocopherol-coated salt in the dry cure. A variation of the curing procedure was also investigated in which the dry cure period was extended from 1 to 7 days, followed by a 3 day immersion in the cover pickle. In all cases, three bellies were used for treatments, i.e. two controls plus two treatments using the α-tocopherol salt. The smoking, slicing and packaging of the bacon samples was completed as previously described.

Mixed tocopherols versus α-tocopherol as N-nitrosamine inhibitors: Dry cured bacon was prepared in the pilot plant as previously described (treatment 3, Table 5) using both 2% α-tocopherol-coated salt and salt coated with mixed tocopherols (Henkel Corp., Minneapolis, MN) at a level such that the effective concentrations of the latter on the salt were 2.5%. The bacon was cured, smoked, sliced and packaged as previously described in the "pilot plant study".

Brine Cured Bacon

Twenty pork bellies (approximately 10-12 pounds) were obtained from a local supplier soon after slaughter and stored for no more than two days in a cooler at 2^oC. The bellies were randomly divided into four groups, and all bellies were stitch pumped to 110% of their green weight with a brine containing 15% sodium chloride (salt), 5.0% sucrose, 3.5% sodium tripolyphosphate, 1200 mg/kg sodium nitrite and 5500 mg/kg sodium ascorbate. The bellies in the control group (treatment 1, Table 6) were pumped with a brine containing regular Alberger Fine Flake salt, while groups 2, 3, 4 and 5 were prepared with salt coated with α-tocopherol. Treatment 2 and 3 consisted of bellies treated with an ingoing level of 250 and 500 mg/kg α-tocopherol, respectively, while bellies in treatment 4 and 5 were prepared using salt coated with both α-tocopherol and lecithin. The latter compound was added to increase the dispersibility of α-tocopherol in the brine. In both treatments, ingoing levels of α-tocopherol and lecithin were 500 and 1000 mg/kg, respectively. The only difference between treatment 4 and 5 was the mode of preparation of the curing brines. In treatment 4, the brine was prepared by adding the curing

Table 6. Target or ingoing concentrations of brine curing ingredients (pilot plant study).

Treatment	Salt (%)	Sugar (%)	Sodium tri-poly phosphate (%)	Sodium nitrite mg/kg	Sodium ascorbate mg/kg	α-tocopherol mg/kg	Lecithin mg/kg
1	1.5	0.5	0.35	120	550	0	0
2	1.5	0.5	0.35	120	550	250	0
3	1.5	0.5	0.35	120	550	500	0
4	1.5	0.5	0.35	120	550	500	1000
5	1.5	0.5	0.35	120	550	500	1000

ingredients to the water individually, while in treatment 4, the brine solution was prepared by mixing all the ingredients together before adding to water. The bellies were smoked, tempered, sliced and packaged as previously described for dry cured bacon.

Feeding Trial

Preparation of the Diet

An α-tocopherol level of 500 mg/kg and 20% corn oil were added to a basic (control) ration as shown in Table 7, and fed *ad libitum* to three groups of three pigs each. The basic diet was a commercial type maize-soybean meal-based diet supplemented with vitamins and minerals to meet nutritional requirements (Table 8). The three different diets were transferred separately to a stainless steel Wenger horizontal mixer (Wenger Mixing Mnfg. Co., Sabetha, KS) and homogeneously mixed with vitamins and mineral supplements. The three lots of feed each weighed 750 kg. The feed was packed in 25 kg bags and stored at room temperature.

Experimental Animals

Nine pigs weighing 66-86 kg were allotted at random into three groups of 3 and were fed the above described diets for 4 weeks. The pigs were housed in pens with aluminum slotted floors in an atmosphere controlled

Table 7. Composition of supplemented diets.

Intredients	Control (lbs) Treatment-I	Vitamin E supplemented (lbs) Treatment-II	High unsaturated (lbs) Treatment-III
Ground shelled corn	631	631	481
Soybean meal	93.75	93.75	93.75
Ground limestone	7.5	7.5	7.5
Mono-dicalcium phosphate	8.25	8.25	8.25
M.S.U. vit-trace mineral mix	3.75	3.75	3.75
Salt	1.9	1.9	1.9
Selenium-vit. E premix[a]	-	-	3.75
Selenium premix[b]	0.38	0.38	-
Vitamin E mix[c]	-	1.36	-
Corn oil	-	-	150
Total	746.53	747.89	749.90

[a]Contains 20 mg/kg Se and 3.4375 IU vitamin E/kg.

[b]Contains 200 mg/kg Se.

[c]Contains 275,000 IU vitamin E/kg.

Table 8. Composition of the basic ration[a,b].

Ingredients	Percentage
Corn-ground	75.35
Soybean meal	21.85
Mineral mixture[c]	2.30
Vitamin premix[d]	0.50

[a]Feed analysis: proteins, 16.5%; lysine, 0.80%; methionine+ cystine, 0.55%; tryptophan, 0.19%; calcium, 0.67%, and phosphorus, 0.505%.

[b]Digestable energy, 3436 Kcal/kg.

[c]Composition of mineral mixture as percentage of diet:sodium chloride, 0.50; limestone, 1.00; dicalcium phosphate, 1.00; and the following in mg/kg: Se, 0.1; Zn, 74.8; Mn, 37.4; I, 2.7; Cu, 9.9; Fe, 59.4.

[d]The vitamin premix supplied the following per kilogram of ration: Vitamin A, 3300 IU; vitamin D, 660 IU; vitamin E, 5.5 IU; vitamin K compound, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; D-pantothenic acid, 13.2 mg; choline, 110.0 mg, and vitamin B_{12}, 19.8 μg.

building. Each pen was equipped with a self-feeder and nipple-type waterer. Feed and water were offered *ad libitum*. The pigs were weighed initially and after 4 weeks of feeding. Feed consumption was also recorded at the end of the feeding trial. The pigs consumed an average of 2.83 to 3.17 kg of feed per day and an average gained 0.77 to 0.93 kg/day.

Slaughtering and Manufacturing of Bacon

The pigs were taken to the MSU Meat Laboratory at approximately 5 p.m. on the day preceeding slaughter. They were held off feed until approximately 6 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. Hams, loins and bellies from both sides of the carcass from the experimental animals were collected.

Eighteen pork bellies (approximately 8-11 pounds) were obtained from the pigs fed with regular, vitamin E (α-tocopherol), and corn oil-supplemented diets, representing 6 bellies per treatment. These bellies were stitch pumped to 110% of their green weight with a brine containing 15% sodium chloride (salt), 5.0% sucrose, 3.5% sodium tripolyphosphate, 1200 mg/kg sodium nitrite and 5500 mg/kg sodium ascorbate. The bellies were equilibrated for 48 hours and smoked, tempered, sliced and packaged as previously described for dry cured bacon. The brine cured bacon manufactured from these pigs was stored for 0, 4 and 8 weeks at refrigerated conditions ($4^{o}C$). The bacon was analyzed for N-nitrosamines, α-tocopherol, residual nitrite, salt, and fatty acid composition. TBA values were also determined.

N-Nitrosamine Analysis

Skillet Calibration

The electric skillet (General Electric Company) was calibrated using a thermocouple attached to a Teflon paddle. Approximately ¼ in. of cooking oil was placed into the skillet. The thermostat was set to $171^{o}C$. The temperature was recorded every minute while stirring in a "figure 8" motion. High and low temperatures were noted and the thermostat was adjusted to produce a minimum temperature of $171^{o}C$. The liquified fat was poured off and any excess was removed with paper towels. The procedure was repeated at least two times daily if bacon were being cooked all day.

Bacon Frying

The skillet was turned on and the temperature allowed to cycle at least 10 min. As many strips of bacon were put into the skillet as possible without overlapping but with room for turning. The bacon was fried on each side for 3 min, removed and drained on paper towels. The cook-out fat was saved and the excess wiped out. The above steps were repeated if the same treatment of bacon was to be cooked. If a different treatment of bacon was used, the skillet was cleaned and calibration steps were then repeated. The fried bacon was stored overnight in a freezer at approximately -30^{o}C.

Extraction of Fried Bacon

The fried bacon was frozen and ground and mixed thoroughly twice prior to analysis. N-Nitrosamines were determined using the gas chromatography-thermal energy analyzer (TEA method of Fine et al., 1975) as modified by Robach et al. (1980), with the exception that ammonium sulfamate (1 g) was added to the distillation flask immediately before distillation commenced. After extraction of the distillate with dichloromethane, the solution was poured onto a PREP-TUBE (Thermal Electron Corporation, Waltham, MA) which had been previously washed with 5x15 ml aliquots of dichloromethane (to remove any N-nitrosamine contamination) and collected in a Kuderna Danish concentrating apparatus fitted with 4 ml receiver. The sample was concentrated and brought to 0.5 ml with dichloromethane, transferred to a 1 ml conical-shaped vial with a teflon-lined cap and placed in a freezer (-20^{o}C).

Extraction of Cook-out Fat

N-Nitrosamines in the cook-out fat were isolated and separated essentially 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

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 a 6' x 1/8" i.d. stainless steel column packed with 10% Carbowax 20 M + 5% KOH on 80/100 mesh Chromosorb W (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, 150^{o}C; GC column temperature, 180^{o}C isothermal; TEA pyrolyzer furnace, 425^{o}C; TEA reaction chamber pressure, 1.5 Torr; TEA attenuation, as appropriate; ice bath temperature, -160^{o}C (isopentane/liquid nitrogen slush bath); GC-TEA heated transfer line, 175^{o}C. The linearity of the response of the GC-TEA was established by injecting the standard N-nitrosamines (Aldrich Chemical Co., Milwaukee, WI) over a range of 0.2-20 ng injected material. All components of the mixed N-nitrosamine standard solution showed linearity over this range. Raw data were collected and processed by a Hewlett Packard Model 3390A reporting integrator. Concentrations of the N-nitrosamine of interest was calculated from the following equation.

$$\mu g/kg = \frac{1000.V.\ x.\ C}{S.W}$$

where V = final volume of extract after concentration, in ml; x = sample TEA detector response of peak area; C = standard concentration, mg/ml; S = standard TEA detector response of peak area; W = weight of bacon sample analyzed in grams.

Percent recoveries of the N-nitrosamines from the fried bacon samples were determined by spiking known amounts of NPYR and NDMA into the distillation flask containing 25 g of fried pork belly prior to distillation. The fried pork belly sample had been previously analyzed and found to contain no NPYR and NDMA. Average recoveries for the spiked samples were 82 and 92% for NPYR and NDMA, respectively. Similarly, percent recoveries of NPYR and NDMA from pork belly drippings spiked with the N-nitrosamines were 76 and 87% respectively.

Mass Spectrometric Confirmation of N-nitrosamines

A Hewlett-Packard 5983A GC-MS unit equipped with a 2 m x 3 mm i.d. glass column packed with 10% Carbowax 20 M + 5% KOH on 80/100 mesh Chromosorb W was used to confirm the identity of N-nitrosamines in samples containing greater than 10 μg/kg of NPYR and NDMA. Operating conditions included: electron energy (EV) 70; actual source temperature, 200^{o}C; helium carrier gas, 25 ml/min.

Chemical Analysis

Proximate Analyses

Proximate analyses (moisture, fat and protein), salt and nitrite determinations were carried out according to standard AOAC procedures (1975). In the nitrite determinations, N-1-naphthylethylene diamine was used instead of α-naphthylamine since the latter compound has been reported to be carcinogenic (Usher and Telling, 1975).

Tocopherol Analysis by HPLC

Essentially, the high performance liquid chromatographic procedure of Thomson and Hatina (1979) was used for tocopherol analysis. Fat extraction and saponification was carried out according to Thomson and Hatina (1979).

A Waters Associates, Inc. (Milford, MA) ALC-201 Model high performance liquid chromatograph equipped with U6K loop injector, Model 440 UV absorption detector (set at 280 nm) and Partisil PXS 10/50 column (50 c.m. x 3 mm; Whatman, Inc., Clifton, NJ) was used for analysis. The solvent system consisted of 1.5% 2-propanol in moist hexane (prepared by mixing equal parts of dry hexane and water-saturated hexane) at a flow rate of 1.5 ml/min. Tocopherols (α and γ) obtained from Eastman Kodak, Co., Rochester, New York, were used as standards. The results were calculated from the peak areas using factors derived by chromatography of above standards. Percent recoveries of the tocopherols from the ground bacon samples were determined by spiking tocopherol-free bacon with known amounts of α-tocopherol before extraction. An average recovery of 76% was obtained.

TBA Values

Thiobarbituric acid (TBA) values were determined on ground bacon samples made from the swine feeding trials at regular intervals during the eight weeks of storage using the method of Tarladgis et al. (1960), as modified by Zipser and Watts (1962). Analyses included four distillations per sample and two colorimetric reactions per distillation. Absorbance was read at 532 nm and TBA numbers (mg malonaldehyde per 1000 g of sample) were calculated using a constant of 7.8.

Fatty Acid Composition by GLC

The fatty acid composition of bacon adipose tissue was determined by gas chromatographic analysis of their fatty acid methyl esters.

Esterification of the fatty acids was carried out according to the procedure of Morrison and Smith (1964) using BF_3-methanol as methylating reagent.

A Hewlett Packard gas chromatograph (Model 5840A) equipped with a flame ionization detector (FID) and Hewlett Packard 18850A GC integrator was used for the analysis of the fatty acid methyl esters. The glass column (2 m x 2 mm. i.d.) was packed with 15% diethylene glycol succinate (DEGS) on Chromosorb W 60/80 mesh. Operating conditions for the system were: GC carrier gas and flow rate, nitrogen at 30 ml/min; GC injection port temperature, 210^{o}C; GC column initial temperature, 190^{o}C and final temperature 260^{o}C; FID temperature, 350^{o}C; attenuation, 8; and chart speed, 1 cm per min. Standard fatty acid methyl esters were prepared under identical conditions and used for identifying and quantitating the fatty acids in the samples.

Clostridium botulinum Study

The various α-tocopherol treated salt-nitrite combinations in the dry cured bacon were examined as to their efficacy in inhibiting *C. botulinum* growth and toxigenesis. A *C. botulinum* spore suspension containing equal numbers of four Type A strains (36A, 52A, 77A and 10755A) and five Type B strains (41B, 53B, 213B, 7949B and Lamanna B) was prepared as described by Rhodes and Jarvis (1976). Spore suspensions of the individual strains were supplied by the University of Minnesota (St. Paul, MN). *C. botulinum* spore counts were determined using the three-tube Most Probable Number (MPN) technique, employing incubation in a TPSY broth (5% trypticase, 0.5% peptone, 1.0% yeast extraction, 0.2% sucrose and 0.1% sodium thioglycolate at pH 7.2 for 7 days at 35^{o}C) (Emodi and Lechovich, 1969). The composite spore suspension was diluted in sterile, distilled water, and heat-shocked at 80^{o}C for 15 minutes.

Sufficient inoculum was spread on one-100 g bacon slices to provide a concentration of 100 spores/g of product. Pork belly slices without nitrite (control) was also inoculated in order to check for the viability of the spore suspension. Inoculated and uninoculated samples from each treatment group were vacuum packaged (Multivac, West Germany) in Vac 4^{R} bags (Koch, Kansas City, MO) and temperature abused or incubated at 27 ± 1^{o}C. Samples were removed after 0, 3, 5, 7, 10, 14, 21, 28 and 42 days when held under abuse conditions and checked for swellings. Three unswollen packages per treatment group were analyzed at each sampling time for toxin development. If packages appeared swollen, they were removed and also analyzed for toxin as further confirmation for the presence of clostridial growth and toxigenesis.

Assays for botulinal toxin were conducted using the procedure of Christiansen et al. (1973). The bacon sample (50 g) was blended with 100 ml of gelatin-phosphate diluent (0.2% gelatin and 0.4% Na_2HPO_4 at pH 6.2). The homogenate was centrifuged and 0.2 ml aliquots of the supernatant were injected intraperitoneally into each of two Swiss Webster white mice weighing approximately 20 g. Death of at least one mouse after the appearance of typical symptoms of botulism during the next 72 hours was considered evidence of the presence of botulinal toxin. Extracts from uninoculated samples stored at 27 ± 1^{o}C were periodically injected into mice as treatment controls, containing 0 mg/kg of α-tocopherol and 120 mg/kg of nitrite.

RESULTS AND DISCUSSION

Inhibition of N-Nitrosamine Formation in Dry Cured Bacon

Pilot Plant Study

Analyses of finished dry cured bacon. The target ingoing levels of curing ingredients for the initial pilot plant study are shown in Table 5, while the levels of ingredients attained in the finished bacon (after slicing) as well as the moisture, fat and protein levels are presented in Table 9. These data reflect good processing control. Residual α-tocopherol levels for the bacon samples treated with 500 mg/kg of α-tocopherol were approximately similar to those reported by Fiddler et al. (1978) for brine-cured bacon, in which the α-tocopherol was dispersed in the brine by the use of Polysorbate emulsifiers.

N-Nitrosamines of dry cured bacon. The effectiveness of α-tocopherol-coated salt as an inhibitor of NPYR formation in fried bacon is shown in Table 10. NPYR concentrations in the range of 36-48 μg/kg (average 42 μg/kg) were obtained for the control samples (treatment 1) to which no α-tocopherol was added. These values are consistent with those cited by Pensabene et al. (1979) who reported NPYR

Table 9. Analysis of finished dry cured bacon (pilot plant study)[a].

Treatment	Nitrite[b] (mg/kg)	α-Tocopherol (mg/kg)	Salt (%)	Moisture (%)	Fat (%)	Protein (%)
1	46	1.0	2.1	33.2	49.4	11.5
2	49	111.0	2.0	33.0	49.4	11.0
3	43	360.5	1.9	31.13	48.5	12.9
4	43	537.5	2.1	33.3	49.2	11.4

[a]Each value represents the average of 3 analyses of 6 samples per treatment.
[b]Nitrite analyses carried out 13 days after initial rub.

Table 10. N-Nitrosamine levels and percent inhibition of nitrosamine formation in dry cured bacon (pilot plant study)[a].

Treatment	NPYR (μg/kg	Inhibition (%)	NDMA (μg/kg)	Inhibition (%)
1	42 (36-48)[b]	-	5 (4-8)	-
2	15 (10-16)	65.3	9 (7-13)	-
3	1.5 (1-3)	96.4	10 (7-18)	-
4	7 (5-9)	83.3	20 (16-25)	-

[a]Each value represents the average of three analyses of six bellies per treatment.

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

levels ranging from 39-89 μg/kg for seven dry cured bacon samples. Similarly, the Nitrite Safety Council (1980) quoted values ranging from trace amounts to 320 μg/kg of NPYR for 15 bacon samples. The addition of 250 mg/kg of α-tocopherol (treatment 2) to the pork bellies resulted in a marked reduction (65%) in NPYR formation. This overall average reduction was obtained by combining all N-nitrosamine extracts for each treatment and expressing it as a percentage of the NPYR in the combined control samples. The introduction of 500 mg/kg of α-tocopherol (average residual 360 mg/kg) into the bacon resulted in almost total inhibition of NPYR formation. This indicates that the application of α-tocopherol to the surface of the rubbing salt is a very effective way of incorporating the blocking agent in the cured bellies. The average percent inhibition (96.4%) of NPYR formation was much greater than the values reported by Fiddler et al. (1978) and Mergens and Newmark (1979). Calculations from the data presented in the latter two publications revealed that up to 85% inhibition of NPYR was obtained.

The higher level of ingoing α-tocopherol (treatment 4, 750 mg/kg) was not as effective as treatment 3 (Table 10) in which the ingoing α-tocopherol level was 500 mg/kg. Pensabene et al. (1978) also found that α-tocopherol when used at a concentration of 1000 mg/liter in an oil-aqueous-protein model system was not as efficient in inhibiting

NPYR formation as the 500 mg/liter formulation. These authors speculated that the higher concentration of Polysorbate emulsifier used in the 1000 mg/liter formulation might be responsible for the reduced effectiveness. In the preparation of the dry cured bacon samples, a 5% α-tocopherol-coated salt system was necessary to achieve the 750 mg/kg ingoing level of α-tocopherol. This salt system tended to be sticky and required dilution with regular fine flake salt to permit the correct ingoing level of α-tocopherol. It was originally thought that the clumping together of this salt system might preclude even distribution of α-tocopherol in the treated pork bellies. However, analysis of the finished bacon showed an average residual α-tocopherol level of 537.5 mg/kg (72% of ingoing) in the bacon samples in treatment 4 (Table 9). It is possible that there is an optimum level of α-tocopherol necessary for N-nitrosamine inhibition in bacon. Further studies should be carried out using ingoing levels of 500, 750 and 1000 mg/kg of α-tocopherol to further evaluate this anomaly.

The effect of adding α-tocopherol to dry cured bacon on N-nitrosamine levels in the cook-out fat is shown in Table 11. Again, bacon prepared with 500 mg/kg of α-tocopherol (treatment 3) exhibited the greatest percent inhibition of NPYR formation in the cook-out fat. This value (92%) is much higher than the percent inhibition

Table 11. N-Nitrosamine levels and percent inhibition of N-nitrosamine formation in cook-out fat of dry-cured bacon (pilot plant study)[a].

Treatment	NPYR (μg/kg)	Inhibition	NDMA (μg/kg)	Inhibition
1	53 (40-68)[b]	-	ND[c]	-
2	13 (11-18)	75	2	-
3	4 (3-7)	92	ND	-
4	5 (4-8)	90	3	-

[a]Each value represents the average of three analyses of six bellies per treatment.

[b]Values in parentheses represent range of nitrosamine levels obtained per treatment.

[c]ND, not detected; limit of detection (0.1 μg/kg).

reported by Fiddler et al. (1978) for cure-solubilized α-tocopherol used at the same concentration. The higher level of α-tocopherol (750 mg/kg ingoing) was almost as effective as the 500 mg/kg treatment in inhibiting NPYR formation in the cook-out fat. The percent reduction in NPYR formation in the cook-out fat obtained in this study compares very favorably with that reported by Bharucha et al. (1980) who cited a 92% reduction in nitrosamine level in bacon treated with 500 mg/kg of ascorbic acid dodecanal acetal in soybean oil when used at a level of 500 mg/kg.

α-Tocopherol was not effective in blocking the formation of NDMA during the frying of bacon (Tables 10 and 11) and generally appeared to increase the levels of NDMA in the fried bacon. This is in slight contrast to the findings of Fiddler et al. (1978) who reported generally reduced levels of NDMA in α-tocopherol-treated bacon. However, the degree of inhibition of NDMA was much smaller than that of NPYR. Walters et al. (1976) studied the effect of antioxidants on the production of volatile N-nitrosamines during the frying of bacon and concluded that α-tocopherol was effective in markedly reducing both NPYR and NDMA in the fried bacon, cook-out fat and condensate, particularly when the bacon was fried in a fat containing 800 mg/kg of α-tocopherol, the amounts of NPYR and NDMA in the vapors produced during frying were reduced by 82 and

62%, respectively.

The greater effectiveness of α-tocopherol in blocking NPYR formation during the frying of bacon is probably due to the fact that the precursors of NPYR reside in adipose tissue (Fiddler et al., 1974; Patterson et al., 1976; Coleman, 1978; Spinelli-Gugger et al., 1981). Bharucha et al. (1979) have further suggested that since both NPYR and NDMA concentrations increase substantially towards the end of the frying process, N-nitrosamine formation during frying of bacon 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 speculated that, during the frying of bacon, nitrous acid is converted into N_2O_3 by continuous removal of water and N_2O_3, in turn, undergoes dissociation at high temperatures (>100°C) to nitric oxide and NO_2. Since nitric oxide is relatively stable, it was concluded that the $NO_2^{\cdot}$ radical can act as the chain initiator and abstract the amino proton from proline to give a radical which combines with the $NO^{\cdot}$ radical to give N-nitrosoproline. Fiddler et al. (1978) have suggested that the reduced effectiveness of α-tocopherol in inhibiting NDMA formation may suggest that a different mechanism operates for the production of NDMA than for NPYR.

Clostridium botulinum study. The efficacy of α-tocopherol on the activity of nitrite in inhibiting

Clostridium botulinum growth and toxigenesis was evaluated by subjecting inoculated bacon slices to temperature abuse at 27^{o}C. Table 12 shows that even after 42 days of temperature abuse at 27^{o}C, bacon manufactured with 0, 250, 500 and 750 mg/kg α-tocopherol and 120 mg/kg sodium nitrite did not exhibit *C. botulinum* growth or toxin production which was indicated by the survival of all mice. After three days of temperature abuse at 27^{o}C, pork slices prepared without nitrite cure had sufficient toxin formation to cause the death of all injected mice, indicating the spores inoculated were viable. These data reveal that α-tocopherol in no way interferes with the activity of nitrite in inhibiting *C. botulinum* growth and toxigenesis. This is in agreement with the results of the studies of Tanaka (1980), in which it was demonstrated that α-tocopherol does not interfere with the antibotulinal activity of nitrite in tests of α-tocopherol-treated bacon conducted shortly after processing.

Small Scale Commercial Study

In order to evaluate the commercial applicability of α-tocopherol coated salts as N-nitrosamine blocking agents, bacon was manufactured by a local small scale commercial operator. The curing process consisted generally of a 24 h dry rub followed by immersion for 9 days in a cover pickle containing 1200 mg of nitrite/liter. A variation of this procedure was also employed in which the dry cure period

Table 12. Effect of α-tocopherol on _C. botulinum_ growth and toxigenesis of dry cured bacon.[a]

Treatment α-tocopherol (mg/kg)	Response of the mice at different time intervals								
	Time interval (days)								
	0	3	5	7	10	14	21	28	42
Pork slice (without nitrite cure)		+							
Regular bacon 0	-	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	-	-	-
500	-	-	-	-	-	-	-	-	-
750	-	-	-	-	-	-	-	-	-

-Indicates survival of mice.

+Indicates death of mice.

[a]All the dry cured bacon was processed with an ingoing level of 120 mg/kg sodium nitrite except where indicated.

was extended to 7 days, followed by a 3 day immersion in the cover brine. Results of analysis of the finished bacon are presented in Table 13. It is apparent that the longer the immersion time in the cover pickle, the greater the residual nitrite and salt levels in the finished products. As expected, the bacon samples prepared by the 24 h dry rub contained less α-tocopherol than those subjected to a 7 day dry rub. This indicated that more time is required for diffusion of α-tocopherol into the pork bellies.

Results of the N-nitrosamine analyses for the commercially prepared bacon are presented in Table 14. As expected, there was a higher average reduction (63%) in NPYR Levels in the bacon samples prepared by the 7 day dry rub. Although the degree of inhibition of NPYR formation in these samples was lower than those obtained in the pilot plant study which may be due to the lower levels of α-tocopherol and higher levels of residual nitrite. However, results indicate that α-tocopherol-coated salt is an excellent medium for incorporating the blocking agent into the pork bellies. Furthermore, this study has shown that considerable reduction of N-nitrosamine levels in bacon can be obtained by α-tocopherol-coated-salt systems in bacon when conditions are naturally conducive to N-nitrosamine formation, i.e., high residual nitrite concentrations.

Table 13. Analysis of finished bacon (commercial study)[a]

Treatment[b]	Residual[c] nitrite (mg/kg)	Residual α-Tocopherol (mg/kg)	Salt (%)
1	230	2.1	3.3
2	215	180.1	3.4
3	120	2.6	2.5
4	100	291.0	2.3

[a]Average of triplicate determinations of three samples per treatment.

[b]Treatment 1: 24 h dry rub, 9 day cover pickle
Treatment 2: 24 h dry rub + α-tocopherol (500 mg/kg ingoing), 9 day cover pickle
Treatment 3: 7 day dry rub, 3 day cover pickle
Treatment 4: 7 day dry rub + α-tocopherol (500 mg/kg ingoing), 3 day cover pickle.

[c]Nitrite analyses carried out 16 days after initial rub.

Table 14. N-Nitrosamine levels in fried bacon (commercial study)[a].

Treatment[b]	NPYR (μg/kg)	Inhibition (%)	NDMA (μg/kg)	Inhibition (%)
1	60 (55-63)[c]		4 (3-5)	-
2	27 (23-31)	55	11 (10-12)	-
3	51 (45-56)		3 (2.5-4.4)	-
4	19 (16-24)	63	9 (8-15)	

[a]Each value represents the average of 3 determination for 3 samples per treatment.

[b]See Table 13.

[c]Values in parentheses represent range of nitrosamine levels obtained per treatment.

Mixed Tocopherols Versus α-Tocopherol

A secondary objective of the present study of dry cured bacon was to compare the relative effectiveness of dl-α-tocopherol and mixed tocopherols (a mixture of α, β, γ and δ-tocopherols) as N-nitrosamine blocking agents. Bacon was prepared under carefully controlled processing conditions, i.e. 120 mg/kg nitrite and 500 mg/kg of tocopherols. Control samples to which no tocopherol was added were also prepared. Residual nitrite and tocopherol levels are presented in Table 15. While residual α-tocopherol levels in the bacon prepared with the α-tocopherol-coated salt were somewhat comparable to those obtained in the initial pilot plant study (Table 9), residual α- and γ-tocopherol levels in the bacon processed with mixed tocopherols are much lower. No explanation as to why this occurred can be given, although it was observed that at the end of the dry cure period, a milky solution was present in the plastic bags containing the cured pork bellies.

Results of the N-nitrosamine analyses for these samples are also given in Table 15. NPYR formation in the α-tocopherol-treated bacon was reduced by 91%, while a reduction of 73% was obtained in the bacon samples containing mixed tocopherols. This lower degree of inhibition is most probably due to the smaller levels of residual tocopherol in the bacon, although it has been reported that

Table 15. N-Nitrosamine, nitrite and tocopherol levels in dry cured bacon (pilot plant study)[a].

Treatment	Residual[b] nitrite (mg/kg)	Residual Tocopherol (mg/kg) α-Tocopherol	Residual Tocopherol (mg/kg) γ-Tocopherol	NPYR (μg/kg)	Inhibition (%)	NDMA (μg/kg)	Inhibition (%)
Control	38.0	2	-	44 (24-56)[c]	-	3 (2-4)	-
α-Tocopherol	45.0	272	-	4 (2-5)	91	8 (5-9)	-
Mixed tocopherols	39.0	52	77	12 (11-13)	73	ND	-

[a]Each value represents the average of triplicate determinations of four samples per treatment.

[b]Nitrite analyses carried out 13 days after the initial rub.

[c]Range of NPYR levels in four samples per treatment.

γ-tocopherol is somewhat less effective than α-tocopherol as a N-nitrosamine blocking agent both _in vitro_ and _in vivo_ (Kamm et al., 1977; Mergens et al., 1978). While all of the tocopherols can reduce a N-nitrosating agent to nitric oxide, only the α-tocopherol, being fully substituted on the aromatic ring, can completely avoid forming a C-nitroso derivative which can partially transnitrosate or even catalyze the N-nitrosation of a secondary amine or amide (Quaife, 1948; Walker et al., 1979). Results of this phase of the study with α-tocopherol were in excellent agreement with those of the previous pilot plant study where 96% inhibition of NPYR in fried bacon was achieved (Table 10).

It can be concluded from this study that α-tocopherol-coated salt, when used as part of the dry cure is a very effective method of inhibiting NPYR formation in dry cured bacon. When bacon is manufactured under carefully controlled processing conditions, almost total inhibition of NPYR in the fried samples can be achieved. In addition, α-tocopherol-coated salt systems provide a marked reduction in NPYR levels in bacon containing high levels of residual nitrite. Therefore, the application of α-tocopherol to pork bellies through the salt appears to be a very practical and simple procedure for lowering the levels of NPYR in fried dry cured bacon. The length of the dry curing period (approximately 10 days) provides sufficient time for the diffusion of α-tocopherol through the pork belly. Mergens and Newmark (1979) have reported that when α-tocopherol was sprayed on the fat side of bacon, approximately 10% of the tocopherol added migrated past the midpoint of the bacon slice after three days storage.

Inhibition of N-Nitrosamine Formation in Brine Cured Bacon

The successful application of α-tocopherol-coated salts in inhibiting NPYR formation in dry cured bacon prompted an investigation into their possible use with brine cured bacon which is the major form of bacon consumed in the United States (National Academy of Sciences, 1981).

Analyses of Finished Brine Cured Bacon

The target ingoing levels of curing ingredients for this study are shown in Table 6 , while the levels of ingredients attained in the finished bacon are presented in Table 16. Salt (average 1.44%) and nitrite (average 33 mg/kg) levels in the finished bacon were comparable to those reported in literature, while residual α-tocopherol levels (average 450 mg/kg for an ingoing of 500 mg/kg) were higher than those obtained in the dry cured bacon study or reported by Fiddler et al. (1978). These latter workers obtained α-tocopherol levels ranging from 232 to 313 mg/kg for two matched pork bellies that had been cut into thirds (brisket, center and flank portions).

Table 16. Analysis of finished brine cured bacon.[a]

Treatment	Nitrite (mg/kg)	α-tocopherol (mg/kg)	Salt (%)
1	34	1.0	1.42
2	31	138	1.48
3	28	458	1.50
4	34	441	1.42
5	38	450	1.38

[a]Each value represents the average of 3 determinations for 5 bellies per treatment.

N-Nitrosamines in Brine Cured Bacon

Results of the N-nitrosamine analysis in fried bacon and cook-out fat indicate a very high degree of inhibition of NPYR formation (Table 17). NPYR concentrations in the range of 14-27 μg/kg (average 19.5 μg/kg) were obtained for the control samples (Treatment 1). These values are in agreement with those reported in the literature (Robach et al., 1980; Theiler et al., 1981; Amundson et al., 1982). The addition of 250 mg/kg of α-tocopherol (treatment 2) to the pork bellies resulted in marked reduction (57%) in NPYR formation. The introduction of 500 mg/kg of α-tocopherol (treatments 3, 4 and 5) effectively blocked the formation of NPYR in both fried bacon (approximately 90% inhibition) and the cook-out fat (97-100% inhibition, Table 17). α-Tocopherol was also effective in blocking NDMA formation in fried bacon, although there was a wider variation (76-92%) in the percent inhibition obtained for treatment 3, 4 and 5. Less inhibition of NDMA formation in the cook-out fat was also observed. These data agree with the observations of Fiddler et al. (1978) who reported generally reduced levels of NDMA in α-tocopherol-treated bacon.

Lecithin was utilized in treatments 4 and 5 to assist in the dispersion of α-tocopherol throughout the curing brine. It was observed during the pumping operation that there was a slight breakdown of the α-tocopherol-salt

Table 17. N-Nitrosamine levels (μg/kg) and percent inhibition of N-nitrosamine formation in brine-cured bacon and cook-out fat.[a]

Treatment	Fried Bacon		Cook-out Fat		Fried Bacon		Cook-out Fat	
	NPYR	Inhibition	NPYR	Inhibition	NPYR	Inhibition	NPYR	Inhibition
1	19.5 (14-27)	-	18.0	-	5.5 (4-6)	-	4.7 (3-6)	-
2	8.4 (7-10)	57	5.6 (3-8)	69	1.8 (1-4)	67	3.0 (1.5-5.0)	35
3	1.8 (1.1-2.9)	91	trace	97	1.1 (ND-2.0)	80	2.5 (1.9-3.4)	46
4	2.5 (1.4-4.2)	87	trace	97	1.3 (1.0-2.2)	76	2.5 (1.1-3.2)	46
5	1.9 (1.6-2.5)	90	ND[c]	100	0.4 (ND-1.2)	92	2.0 (1.6-2.8)	57

[a]Each value represents the average of three analyses of five bellies per treatment.

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

[c]ND, not detected; limit of detection, 0.1 μg/kg.

system that did not contain lecithin (treatment 2 and 3). This was reflected in the formation of an oily film on top of the brine solution towards the end of the pumping operation. No such effect was observed when lecithin was added to the α-tocopherol-salt systems. The circulating action of the pump used for stitch pumping the bellies was sufficient to keep the α-tocopherol dispersed in the brine solution.

The inclusion of lecithin as part of the α-tocopherol-salt system was initially viewed with some concern as it has been suggested that lecithin may be a precursor of NDMA in fried bacon. Model system studies by Pensabene et al. (1975) and Gray et al. (1978) revealed that NDMA could be formed from lecithin when heated with nitrite. However, results of the present study indicate that lecithin does not contribute to NDMA formation in bacon. This conclusion is supported by the data of Spinelli-Gugger et al. (1981) who observed that the major precursor(s) of NDMA in bacon is/are not extractable with chloroform and is/are thus water-soluble component(s). The nature of these precursors remains undetermined.

Sensory Analysis of Brine Cured Bacon

Sensory evaluation of the flavor of brine cured bacon was carried out by panels composed of twenty students and staff members from the Food Science and Human Nutrition

Department at Michigan State University. The data obtained from triangle tests indicate that 95% ($p = 0.05$) of the panelists were unable to detect any significant differences between the control bacon samples and bacon pumped with brine containing α-tocopherol and lecithin-coated salts.

Feeding Trial Study

Analysis of Finished Bacon

The levels of ingredients attained in the finished bacon as well as the residual α-tocopherol and TBA values are presented in Table 18. These data reflect an average residual nitrite level of 55 mg/kg and salt level of 1.29%. Residual nitrite levels decreased to an average of 23 mg/kg and 7 mg/kg when the bacon was stored under refrigerated conditions for 4 and 8 weeks, respectively. These values are comparable with the earlier studies of Hill et al. (1973), Cassens et al. (1978), Cassens et al. (1979) and Robach et al. (1980), in which it was reported that the residual nitrite levels depleted 50-77% over a period of 31 days storage at a temperature of $5 \pm 2^{o}C$. Residual α-tocopherol levels for the bacon samples manufactured from the pigs fed with 500 mg/kg α-tocopherol-supplemented diets averaged 18 mg/kg. This value compares favorably with the earlier findings regarding the slow absorption of α-tocopherol into the pork adipose tissue (Astrup, 1973;

Table 18. Analysis of finished bacon[a] (feeding trial study).

Treatment[b]	Nitrite (mg/kg)			α-tocopherol (mg/kg)			TBA No. (mg/kg)			Salt (%)		
	0 day	4 wks	8 wks	0 day	4 wks	8 wks	0 day	4 wks	8 wks	0 day	4 wks	8 wks
I	58	21	7	0.5	NA	NA[c]	0.23	0.24	0.40	1.3	NA	NA
II	50	24	5	18	NA	NA	0.18	0.17	0.37	1.26	NA	NA
III	56	23	8	1.0	NA	NA	0.27	0.39	0.52	1.32	NA	NA

[a]Each value represents the average of three analysis of three bellies per treatment.

[b]Treatment I: pigs fed with regular diet.
Treatment II: pigs fed with 500 mg/kg α-tocopherol-enriched diet.
Treatment III: pigs fed with 20% corn oil supplemented diet.

[c]NA, not analyzed.

Hvidsten and Astrup, 1963; Tsai et al., 1978). Tsai et al. (1978) reported that dietary supplementation of 200 mg/kg α-tocopherol acetate results in the deposition of approximately 5.0, 7.7 and 4.3 mg/kg of α-tocopherol/kg tissue in the subcutaneous fat, leaf or renal fat and triceps muscle of pigs, respectively.

TBA Values

Table 18 illustrates the influence of α-tocopherol (Vitamin E) and corn oil supplementation on TBA values, i.e., the concentration of malonaldehyde (mg/kg), produced as a result of oxidation of lipids, particularly of polyunsaturated fatty acids. The low TBA values of bacon prepared from all three different treatments reflect the role of nitrite as an antioxidant (Pearson et al., 1977; Fooladi et al., 1979). Bacon from the α-tocopherol-enriched group had similar TBA values to those of the controls. These values agree with those of Buckley and Connolly (1980) who also showed that the effects of α-tocopherol supplementation on bacon stability are not pronounced, probably due to the presence of nitrite. However, bacon from the corn oil-supplemented group had higher TBA values compared to the control bacon and bacon from the α-tocopherol treatment.

N-Nitrosamine in Bacon

Results of the N-nitrosamine analyses (Table 19) indicate NPYR concentrations in the range of 23-26 μg/kg

Table 19. N-Nitrosamine levels in fried bacon[a] (feeding trial study).

Treatment	NPYR (μg/kg)			NDMA (μg/kg)		
	0 day	4 wks	8 wks	0 day	4 wks	8 wks
1	25 (23-26)[b]	3 (ND-8)	1.5 (ND[c]-3.0)	13 (ND-30)	3 (ND-6)	ND
2	16 (ND-26)	2 (ND-3)	2 (ND-2)	18 (13-20)	5 (2-8)	Tr[d]
3	30 (25-37)	3 (ND-5)	3 (ND-4)	81 (51-113)	9 (8-11)	4 (tr-6)

[a]Each value represents the average of three analysis of three bellies per treatment.

[b]Values in parenthesis represent range of N-nitrosamine levels obtained per treatment.

[c]ND, not detected; limit of detection, 0.1 μg/kg.

[d]Tr, trace amounts; less than 0.5 μg/kg.

(average 25 μg/kg) for the control samples (treatment 1). These values are consistent with those of the earlier brine cured bacon study. Bacon, from the α-tocopherol-enriched group, contained smaller levels of NPYR (average 16 μg/kg) which may be due to low residual levels of α-tocopherol (Treatment 2, Table 18). On the other hand, bacon from the corn oil-supplemented group averaged 30 μg/kg, indicating that corn oil supplementation does not have any apparent effect on NPYR formation.

The concentrations of NDMA obtained for the control samples (treatment 1) averaged 13 μg/kg, while the bacon made from pigs fed the α-tocopherol enriched diet contained slightly higher levels of NDMA (average 18 μg/kg). However, bacon from treatment 3 (highly unsaturated fat, 20% corn oil diet) contained higher levels of NDMA, which ranged from 51-113 μg/kg (average 81 μg/kg). These findings are very interesting in terms of the possible relationship of unsaturated fatty acids and lipid oxidation to the mechanism of NDMA formation. These results may be possibly explained in terms of the nature of the interaction of nitrogen dioxide with the double bonds of unsaturated fatty acids, especially $C_{18:1}$, $C_{18:2}$ or $C_{18:3}$ (Pryor and Lightsey, 1981) and resulting in pseudonitrosites. This unexpected reaction

$$NO_2 + -HC = CH\text{-}CH_2 \longrightarrow NOHO + -CH\text{-}CH\text{-}CH$$

leads to the production of nitrous acid (HONO) rather than the formation of a product containing a nitro group attached to a carbon atom. This is in agreement with the results of Goutefongea et al. (1976) and Walters et al. (1979). In these studies, pseudonitrosites obtained by reaction of nitrogen oxides (Walters et al., 1979) and labelled sodium nitrite (Goutefongea et al., 1976) with alkenes (including unsaturated fatty acids) were shown to be capable of nitrosating secondary amines in a lipid solvent. This may have some relevance to the high levels of linoleic acid ($C_{18:2}$) in the adipose tissue of bacon made from the corn oil-supplemented group (Table 20). It can also be speculated from these experiments that the levels of NDMA in bacon may increase substantially when the system contains higher amounts of unsaturated fatty acids (especially oleic and linoleic acids) via the formation of actively nitrosating pseudonitrosites. These results and the proposed behavior of nitrogen oxides as simple, free-radical initiators are consistent with the model system studies of Pryor and Lightsey (1981), in which dicyclohexylamine (10^{-3}M) was added to $C_{18:1}$ and $C_{18:2}$ fatty acid esters and exposed to 60 mg/kg of nitrogen dioxide in air. Results from this study show that the rate of nitrosation of amine increases with increasing number of double bonds in the reaction medium, an indication that nitrous acid (HONO) formed by the NO_2-unsaturated ester reactions is participating in

Table 20. Fatty acid composition of bacon adipose tissue[a] (feeding trial study).

Fatty acid	Treatment 1 (%)	Treatment 2 (%)	Treatment 3 (%)
Saturated			
$C_{16:0}$	23.54	24.17	17.59
$C_{18:0}$	11.01	12.18	6.38
Unsaturated			
$C_{16:1}$	2.98	2.81	2.09
$C_{18:1}$	44.20	44.90	46.50
$C_{18:2}$	14.85	14.66	27.32
$C_{18:3}$	0.85	0.98	1.04

[a]Each value represents the average of three analysis of three bellies per treatment.

the nitrosation of the amine. This formation of HONO by NO_2-alkene reactions adds credence to the postulate that the unsaturated fats of food may be involved in the nitrosation of amines.

Low levels of NPYR and NDMA were also observed (Table 19) in bacon stored for 4 and 8 weeks at 4^oC irrespective of the treatments. This may very well be correlated to the depletion of nitrite ranging from 50-88% (Table 18). However, somewhat higher levels of NDMA were again obtained in bacon from corn oil treatment compared to control and α-tocopherol-enriched bacon.

Fatty Acid Composition of Bacon Adipose Tissue

The influence of dietary supplementation of α-tocopherol and corn oil on the fatty acid composition are summarized in Table 20. As expected, there were little or no effects of supplementation of α-tocopherol on the fatty acid distribution nor on the amount of unsaturation. The observations thus are in agreement with those of Hvidsten and Astrup (1963) and Tsai et al. (1978) who reported that the degree of unsaturation of body fat is unaffected by supplementation of α-tocopherol. In addition, Bunnell et al. (1956) concluded that there is little evidence to indicate an effect of vitamin E on the overall fatty acid composition of the tissues.

Dietary supplementation with corn oil has a marked effect on the fatty acid distribution in adipose tissue. Corn oil tended to decrease the $C_{16:0}$ and $C_{18:0}$ levels and to increase the $C_{18:1}$ and $C_{18:2}$ levels in the adipose tissue. Specifically, there was an approximate two fold increase in the $C_{18:2}$ content in bacon from the corn oil treatment. These values correlate well with the higher levels of NDMA (Table 19), an indication that increasing levels of $C_{18:1}$ and $C_{18:2}$ formed by the supplementation of corn oil in the bacon may enhance the formation of NDMA.

SUMMARY AND CONCLUSIONS

In this study, the effects of α-tocopherol-coated salt systems on the inhibition of N-nitrosamine formation were investigated in both dry cured and brine cured bacon. In another phase of this investigation, the possible relationship between unsaturated fatty acids in the adipose tissue of bacon and N-nitrosamine formation was evaluated by swine feeding trials in which pigs were fed regular, α-tocopherol-enriched and highly unsaturated fat diets.

N-Nitrosamines (NPYR, NDMA) were determined using a gas chromatograph equipped with a thermal energy analyzer (TEA) detector. These results were further confirmed by mass spectrometry when N-nitrosamine levels greater than 10 μg/kg were obtained. α-Tocopherol levels were estimated using high performance liquid chromatography. The bacon samples were also evaluated for Clostridium botulinum toxigenesis, residual nitrite, fatty acid composition, TBA numbers, salt and by other proximate analyses.

In the first phase of the study, α-tocopherol-coated salt, when used as part of the dry cure, reduced NPYR levels in the fried product by approximately 96% at an ingoing α-tocopherol level of 500 mg/kg. NPYR levels in the cook-out fat of dry cured bacon were reduced approximately 92% using the same level of α-tocopherol. Dry cured bacon prepared by a small scale commercial operator also contained lower levels of NPYR when processed with α-tocopherol-coated salt (500 mg/kg ingoing level), despite the high levels (>100 mg/kg) of residual sodium nitrite in the finished bacon. Results comparing the effectiveness of α-tocopherol and mixed tocopherols as N-nitrosamine blocking agents were inconclusive. Although α-tocopherol appeared to be the more effective as a blocking agent (91% versus 73% inhibition of NPYR), the difference in the percent inhibition achieved could possibly be attributed to the apparently lower levels of residual mixed tocopherols in the finished bacon. It was also observed in this phase of the study, that dry cured bacon when treated with α-tocopherol-coated salt contained higher levels of NDMA compared to the regular (control) bacon.

In the second phase of the study, bacon was made under carefully controlled processing conditions in a pilot plant by pumping brine containing α-tocopherol- and lecithin-coated salts. Results of the N-nitrosamine analyses indicate a very high degree of inhibition of NPYR formation in both fried bacon (approximately 90%) and the cook-out fat (97-100%). α-Tocopherol was also effective in minimizing NDMA formation in fried bacon when applied at an ingoing level of 500 mg/kg. However, there was a wider variation

(76-92%) in the percent inhibition obtained for NDMA levels. Less inhibition of NDMA formation in the cook-out fat was also observed.

In the third phase of the study, feeding trials were conducted in which pigs were fed regular, α-tocopherol-enriched and highly unsaturated fat diets. No obvious effects on the formation of NPYR were observed in bacon manufactured from these pigs. However, bacon, made from swine fed the corn oil-supplemented diets contained much higher levels of NDMA (an approximate six fold increase) than the control bacon i.e. bacon prepared from hogs on the regular diet.

As a result of these investigations, several conclusions pertaining to the inhibition and the mechanism of N-nitrosamine formation in bacon can be reached. These are summarized as follows: (1) α-Tocopherol-coated salt, when used as part of the dry cure is a very effective method of inhibiting NPYR formation in dry cured bacon. When bacon is manufactured under carefully controlled processing conditions, almost total inhibition of NPYR in the fried sample can be achieved; (2) α-Tocopherol-coated salt systems provide a marked reduction in NPYR levels in bacon containing high levels of residual nitrite as illustrated by the small scale commercial study; (3) The results comparing the effectiveness of α-tocopherol and mixed tocopherols as N-nitrosamine blocking agents were inconclusive;

(4) The application of α-tocopherol to pork bellies through the salt appears to be a very practical and simple procedure for lowering the levels of NPYR in fried dry cured bacon. This is in compliance with the recommendation of the Nitrite Safety Council (1980), that the evaluations of cure formulation changes or process control adjustments which may reduce or eliminate N-nitrosamine formation in dry cured bacon are necessary; (5) α-Tocopherol-coated salt system was not as effective in blocking the formation of NDMA during the frying of dry cured bacon and generally increased the levels of NDMA in the fried bacon; (6) Pumping of a brine containing α-tocopherol and lecithin-coated salt into the pork bellies is a very effective method of inhibiting NPYR and NDMA formation in fried brine cured bacon as well as cook-out fat; (7) Overall, it has been demonstrated that α-tocopherol and α-tocopherol, lecithin-coated salt systems are very effective inhibitors of N-nitrosamine formation in fried bacon. These salts can be applied to both dry cured and brine cured bacon products without any changes in current processing procedures. Thus, the application of such systems in bacon processing can be considered as a safe, effective and viable approach to reducing N-nitrosamine levels in cooked bacon; (8) Results obtained from the swine feeding trial study indicate that the presence of higher amounts of unsaturated fatty acids in bacon may enhance the formation of NDMA possibly through

the formation of actively nitrosating pseudonitrosites; (9) α-Tocopherol-enriched and corn oil-supplemented diets do not have a marked effect on the production of NPYR in fried bacon.

PROPOSALS FOR FURTHER RESEARCH

The study of the potential of α-tocopherol-coated salt as a means of suppressing N-nitrosamine formation in bacon, and results of the feeding trials with α-tocopherol and corn oil-supplemented swine rations raise some important questions which merit further study. These include investigations of:

(1) The influence of higher levels of α-tocopherol (750, 1000 and 1500 mg/kg) on the inhibition of N-nitrosamine formation in bacon.

(2) The further investigation of the effects of α-tocopherol-coated salt system on the formation of NDMA in dry cured bacon.

(3) The potential of α-tocopherol-coated salt systems on the inhibition of N-nitrosamines in other dry cured meat products.

(4) The stability of α-tocopherol on the surface of the salt during commercial storage.

(5) The chemical nature and toxicological evaluation of α-tocoquinone, which is a major end product of the interaction of α-tocopherol and residual nitrite during frying of bacon.

(6) The mechanism and various precursors of NDMA formation in cured meat systems.

(7) Further clarification of the influence of unsaturated and saturated fatty acids on the formation of N-nitrosamines by feeding tallow and coconut meal-supplemented diets to the swine rations.

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