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thesis entitled

FORMATION OF N-NITROSOPYRROLIDINE IN FRIED BACON:
MODEL SYSTEM STUDIES

presented by

Man-Lai Lee

has been accepted towards fulfillment
of the requirements for

Master degree in Food Science

J. I. Gray
Major professor

Date August 6, 1981

FORMATION OF N-NITROSOPYRROLIDINE IN FRIED BACON: MODEL SYSTEM STUDIES

By

Man-Lai Lee

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1981

ABSTRACT

FORMATION OF N-NITROSOPYRROLIDINE IN FRIED BACON: MODEL SYSTEM STUDIES

By

Man-Lai Lee

The kinetics of reactions such as the N-nitrosation of proline or pyrrolidine, and the decarboxylation of proline or N-nitrosoproline in heat-induced model systems simulating the pan-frying conditions of bacon were determined. The rate constants, half-life values and activation energy values of these reactions were calculated. Evidence presented strongly implies that the major pathway of N-nitrosopyrrolidine 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 formation of N-nitrosopyrrolidine was affected by parameters such as heating time and temperature and the compositional variation of samples. The internal temperatures attained in bacon samples during frying were significantly higher in the samples fried in a preheated skillet compared to those fried in an initially cold skillet.

Dedicated to

My Family and My Husband

ACKNOWLEDGMENTS

The author wishes to express her sincere thanks and deepest gratitude to Dr. J.I. Gray for his guidance, assistance and friendship in the course of her graduate study.

Genuine appreciation is extended to the members of the research guidance committee, Drs. L.R. Dugan, Jr., R.A. Merkel and J.F. Price of the Department of Food Science and Human Nutrition, and Dr. L.R. Shull of the Department of Animal Science, for reviewing this manuscript.

The author is grateful to the Department of Food Science and Human Nutrition for financial assistance.

A special thank you is given to the author's friends and colleagues for their friendship and encouragement.

Finally, the author wishes to express her heartfelt gratitude to her family and husband for their love, enthusiasm and many sacrifices.

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INTRODUCTION

The consistent isolation of carcinogenic volatile N-nitrosopyrrolidine (NPYR) in fried bacon has prompted researchers to intensify the search for both the precursors and mechanism(s) leading to the formation of this N-nitrosamine.

Free proline (PRO), present in raw side bacon in the range 20-80 mg/kg has recently been shown to be the most probable precursor of NPYR in bacon (Hwang and Rosen, 1976; Gray and Collins, 1977a; Bharucha et al., 1979). While this aspect of NPYR formation has been well established, its mode of formation has yet to be unravelled. Two pathways have been proposed: one involving the N-nitrosation of PRO followed by the decarboxylation of N-nitrosoproline (NPRO) to NPYR (Hwang and Rosen, 1976; Bharucha et al., 1979); the second involving the decarboxylation of PRO to pyrrolidine (PYR) which is then N-nitrosated to produce NPYR (Nakamura et al., 1976; Coleman, 1978).

There have been conflicting reports describing the precise role of NPRO in bacon. Although it has been shown that NPRO is not the primary precursor of NPYR (Hansen et al., 1977; Janzowski et al., 1978; Pensabene et al., 1979b), its involvement in the intermediacy of NPYR formation

still requires further resolution (Bharucha et al., 1979).

The elucidation of the mechanism of formation of NPYR, is therefore, the foremost element of the present study. The kinetics of the N-nitrosation of PRO or PYR and the decarboxylation of PRO or NPRO will be examined. Additionally, some factors influencing the formation of NPYR in fried bacon will be investigated.

LITERATURE REVIEW

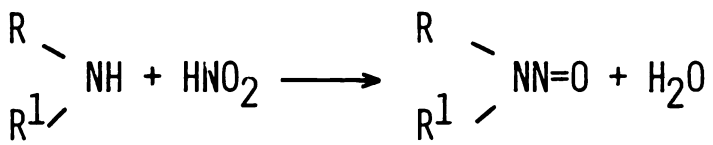
Few topics in the past decade have generated as much discussion and research as the presence of N-nitroso compounds in food systems. More than 80 percent of 130 tested N-nitroso compounds have been shown to be carcinogenic in experimental animals such as monkeys, rats, mice, guinea pigs, rabbits, sheep and some subhuman primates; in addition, some exhibit mutagenic, teratogenic or embryopathic properties (Preussmann et al., 1976; Gray and Randall, 1979; Wishnok, 1979). It is, therefore, highly probable that some of this class of compounds are hazardous to man.

Traditionally, human exposure to N-nitroso compounds was confined to those associated with cured meat products. However, it has since become apparent that they 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 (Sander, 1967; Sen et al., 1969; Lijinsky et al., 1970), thus adding another dimension to the problem. However, nitrite-preserved foods, especially bacon, have continued to be the center of many research activities.

The formation and occurrence of N-nitrosamines, and their toxicological and human health hazards have been adequately documented (Scanlan, 1975; Crosby and Sawyer, 1976; Gray and Randall, 1979; Sen, 1980). In this review, the main focus will be on the formation and occurrence of N-nitrosamines in cured meat products. However, in an attempt to put this subject in perspective, several related areas will be mentioned.

Chemistry of Formation

a. What are N-nitrosamines? N-Nitrosamines are formed principally from the reaction between secondary amines and nitrous acid. In this reaction, R^1 is an alkyl group while



R may be an alkyl, aryl or a wide variety of other functional groups. As will be discussed later, N-nitrosamines can also be formed from tertiary amines, quarternary ammonium compounds and primary polyamines. Many of these N-nitroso compounds have been identified in various food systems including cured meat products, non-fat dried milk, and dried malt and beer (Gray and Randall, 1979). In addition, the presence of less volatile and non-volatile N-nitroso compounds in foods have been suggested from a number of model system studies (Gray and Randall, 1979).

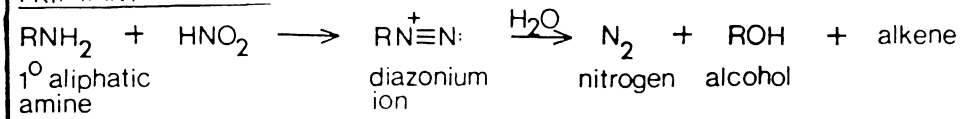
The chemistry of the reactions of amines with nitrous acid leading to the formation of N-nitrosamines has been studied at length. Detailed discussions of this subject matter are given in these papers (Mirvish, 1972; 1975; Scanlan, 1975; Crosby and Sawyer, 1976).

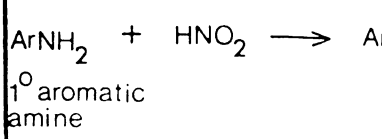
b. N-Nitrosation reactions. When a primary amine reacts with nitrous acid (Figure 1), an unstable intermediate (diazonium ion) is formed which then loses nitrogen to produce a carbonium ion. The carbonium ion can undergo further addition, elimination or rearrangement to produce a variety of deamination products. On the other hand, primary aromatic amines react with nitrous acid to yield stable diazonium salts (Morrison and Boyd, 1974).

Secondary amines, both aliphatic and aromatic, react with nitrous acid to produce stable N-nitrosamines (Figure 1). Tertiary aliphatic amines (and to a lesser extent, tertiary aromatic amines, particularly if the p-position is blocked) react with nitrous acid to yield a N-nitroso derivative of secondary amine (Figure 1). The first step is similar to the N-nitrosation of primary or secondary amines in that the lone pair of electrons on the unprotonated amine reacts with a N-nitrosating species. The nitrosammonium ion formed undergoes cis-elimination of the nitroxyl ion to form an immonium ion which is hydrolyzed to a carboxyl ion, aldehyde or ketone and, secondary amine. The secondary amine is N-nitrosated to the corresponding N-nitrosamine

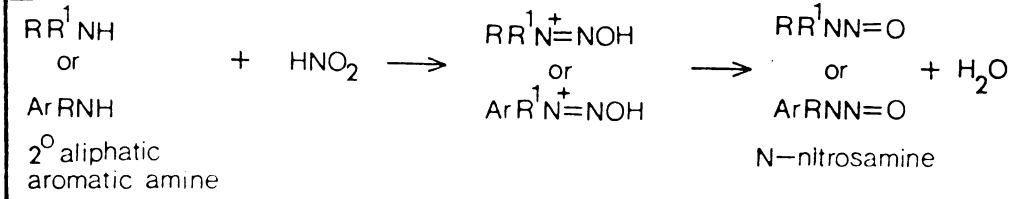
Figure 1. Reactions of primary, secondary and tertiary amines with nitrous acid.

PRIMARY AMINES

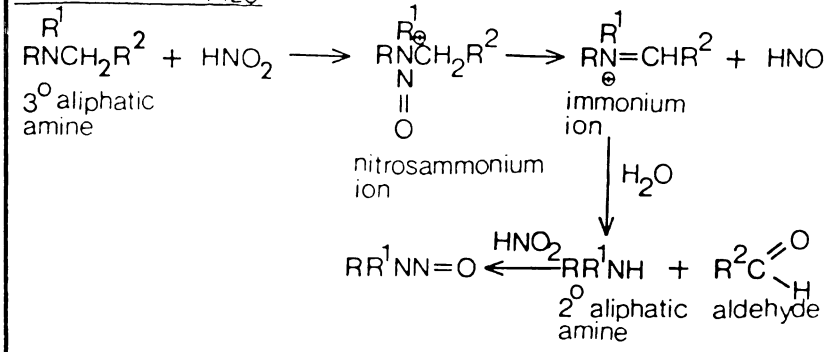


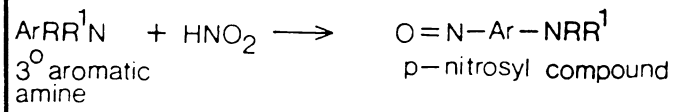


SECONDARY AMINES



TERTIARY AMINES



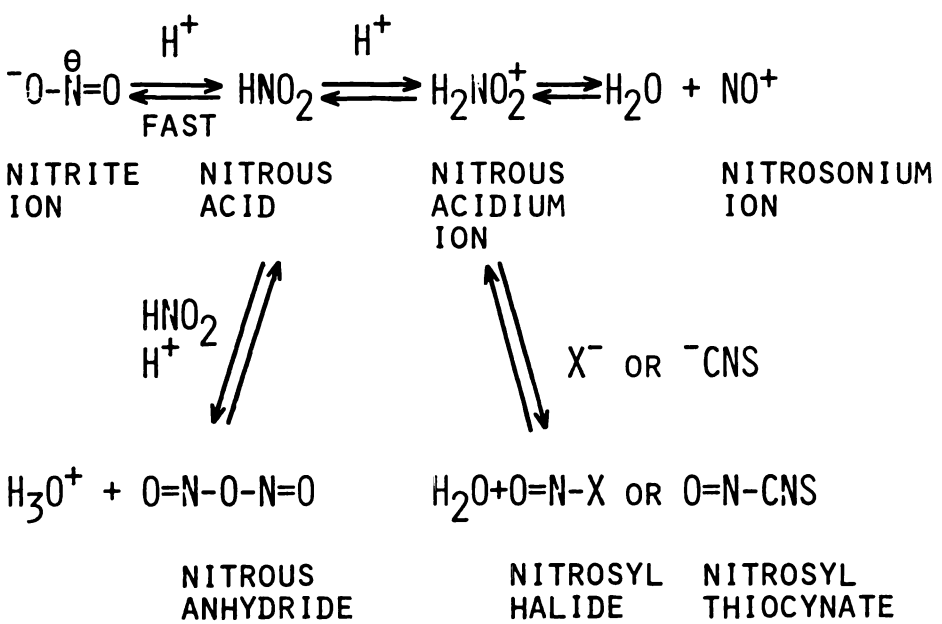


(Smith and Loeppky, 1967).

In more complex food systems, N-nitrosamines can also be formed from primary amines, albeit in low concentrations (Scanlan, 1975). Primary diamines or diamino acids with chains of four or five carbon atoms, such as putrescine, can cyclize quite readily to a cyclic secondary amine and much higher yields of N-nitrosamines can be obtained. Complex biological polyamines, such as spermidine, can similarly give rise to N-nitroso derivatives of cyclic amines and to a variety of N-nitrosamines with substituted dialkyl groups. Quarternary ammonium compounds can also react with nitrous acid to produce N-nitrosamines, although the yields are much lower than those from secondary or tertiary amines (Fiddler et al., 1972). More recently, Pensabene et al. (1975) and Gray et al. (1978) showed that N-nitrosodimethylamine (DMN) could be produced from lecithin-type compounds under various conditions.

The extent of formation of N-nitrosamines from N-nitrosatable amines is governed by a variety of factors such as the basicity of amine, concentration of reactants, pH, temperature and the presence or absence of catalysts and inhibitors. A clear concept of the kinetics of N-nitrosation reaction is, undoubtedly, an essential element for studying the formation of N-nitrosamines in foods as well as in the human stomach and intestines.

c. Kinetics of the N-nitrosation reaction. The kinetics of N-nitrosamine formation from secondary amines and nitrous acid has been studied in detail by Mirvish (1972; 1975). For N-nitrosation to occur, nitrite is first converted to nitrous acid (HNO_2) (pK_a = 3.36) which is then converted to an active N-nitrosating species. This explains why the reaction is catalyzed by acid. The actual N-nitrosating species can be one of the following, depending on the reaction conditions: nitrous anhydride (N_2O_3), nitrous acidium ion ($H_2NO_2^+$), free nitrosonium ion (NO^+), nitrosyl halide (NOX) or nitrosyl thiocyanate (NOCNS) as shown below (Ridd, 1961; Challis and Butler, 1968; Mirvish, 1975).



For most secondary amines in meat systems, N-nitrosation reactions proceed via the active N-nitrosating species, nitrous anhydride. Since low pH is not encountered in meat

systems, the nitrous acidium ion and the free nitrosonium ion cannot be considered as important N-nitrosating species. However, Keefer (1976) reported that nitric oxide (NO) can bring about the N-nitrosation of secondary amines, particularly at alkaline pH and in the presence of copper and iron salts as catalysts. Assuming N-nitrosation was via nitrous anhydride, Mirvish (1975) proposed the overall third order rate equations as follows:

$$\text{rate of N-nitrosamine formation} = k_1(R\ R^1NH)(HNO_2)^2 \quad \ldots\ldots\ldots \quad (1)$$

$$\text{rate of N-nitrosamine formation} = k_2\ (\text{total amine})(\text{nitrite})^2. \quad (2)$$

where k_1 and k_2 are the respective rate constants. Thus, the N-nitrosation reactions are first order with respect to amine concentration and second order with respect to nitrite concentrations.

In equation (1), the concentrations expressed are those of unprotonated amine and undissociated nitrous acid (both of which are pH dependent), and k_1 is independent of pH. However, in equation (2), the total concentrations are used, and k_2 varies with pH and shows a maximum value at pH 3.0-3.4 (Mirvish, 1975). In the pH range of 5-9, the rate of N-nitrosation of dimethylamine was found to increase by 10 fold for each decrease of one pH unit (Mirvish, 1970).

Since the concentration of a nonionized (unprotonated) secondary amine is inversely proportional to the basicity of

the amine, the rate constant (k_2) of N-nitrosation of weakly basic amines would be higher than that of strongly basic amines. This is borne out by the results of Mirvish (1975) who studied the kinetics of the N-nitrosation reaction of various secondary amines and amino acids (Table 1).

At optimum pH, the relative rate of N-nitrosation of the least basic secondary amine, piperazine (pK_a = 5.57), is about 185,000 faster than that of piperidine (pK_a = 11.2) and about 50,000 times faster than dimethylamine (pK_a = 10.72). Similar results were previously obtained by Sander et al. (1968). For weakly basic amines, the concentration of unprotonated amines may increase to the extent that the reaction rates become independent of the amine concentration. The reaction then follows the second order kinetics as follows:

$$\text{rate of N-nitrosamine formation} = k(HNO_2)^2 \quad \ldots\ldots\ldots (3)$$

The basicity of amine, the concentration of nitrite and pH are, therefore, the three most important factors influencing the formation of N-nitrosamines.

Similar kinetic studies (Mirvish, 1972) with N-nitrosatable amino acids such as PRO, hydroxyproline and sarcosine, indicate that the optimum pH of N-nitrosation is about 2.25-2.50. On the other hand, N-nitrosation of akylureas and akylurethanes (Mirvish, 1972; Sander and Schweinsberg, 1972) does not exhibit a maximum at any pH. In the pH

Table 1. Rate constants for the N-nitrosation of amines at the optimum pH and 25°C[a].

Amine	pK_a	Optimum pH	k_2[b] (M^{-2} sec^{-1})
Piperidine	11.20	3.0	0.00045
Dimethylamine	10.72	3.4	0.0017
Pyrrolidine	11.27	3.0	0.0053
N-Methyl-ethanolamine	9.50	3.2	0.0010
N-Methyl-benzylamine	9.54	3.0	0.013
Proline	--	2.5	0.037
Sarcosine	--	2.5	0.23
Propylglycine	8.97	3.0	0.25
Hydroxyproline	--	2.5	0.31
Propylleucyl-glycineamide	8.97	3.4	0.38
Morpholine	8.70	3.4	0.42
Mononitroso-piperazine	6.80	3.0	6.7
Aminopyrine	5.04	2.0	80
Piperazine	5.57	3.0	83
N-Methylaniline	4.85	--	250

[a]Adapted from Mirvish (1975).

[b]pH dependent rate constant from rate equation (2).

range of 3 to 1, the rate of N-nitrosation for akylureas and akylurethanes increases about 10 fold for each pH unit decrease. Furthermore, in contrast to the situation with the amines or amino acids, the rate is proportional to the concentration of nitrite and not its square.

As in the case with all chemical reactions, temperature has a pronounced effect on the rate of N-nitrosation. For every 10^{o}C rise in temperature, the reaction rate is doubled (Foreman and Goodhead, 1975). Ender et al. (1967) studied the effect of temperature and storage on the formation of DMN from 40 mM each of dimethylamine hydrochloride and sodium nitrite buffered at pH 6.5. The results obtained suggest that heating of nitrite-containing foods under reflux for 15 min and prolonged storage at 4^{o}C may substantially accelerate the formation of N-nitrosamines. Later, Ender and Ceh (1971) reported that N-nitrosation reaction also occurs at a steady rate in the temperature range of -18 to -37^{o}C. At sub-freezing temperatures, an enhancement of N-nitrosation occurs because the solutes become concentrated in the liquid phase.

Precursors of N-Nitrosamines in Food Systems

a. Nitrates and nitrites. Nitrate is present virtually in all biological materials and constitutes the primary source of fixed nitrogen in green plants. It occurs in high concentration (sometimes as high as 1,000 to 3,000 mg/kg) in

vegetables (Ashton, 1970; White, 1975; Lin and Lue, 1979; Lin and Yen, 1980). The levels of nitrate present in vegetables depend mainly on the nitrate-supplying power of the soil, the genetic makeup of the vegetable and the growing condition (Brown and Smith, 1966; Maynard et al., 1976). Nitrate also occurs in water, especially in well waters, in some rural areas (Comly, 1945; Burden, 1961). 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). Lin and Yen (1980) also reported an increase in nitrite concentration in some Chinese vegetables during the first few days of storage at 26 or 32^{o}C, while the nitrate concentration decreased. Little change in the nitrate and nitrite concentrations was observed during storage at -10 or 2^{o}C.

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). Nitrate per se does not have any inhibitory action against these bacteria, but its action is manifested by the reduction of nitrate to nitrite

by microorganisms present in foods. Apart from their preserving action, these chemicals are believed to play an important role in (a) producing the characteristic cured meat color (nitrosyl hemochrome) (Brooks et al., 1940; Dryden and Birdsall, 1980); (b) contributing the characteristic cured meat flavor (Bailey and Swain, 1973) and (c) providing antioxidant effects and eliminating the problem of warmed-over flavor (Bailey and Swain, 1973; Pearson et al., 1977; MacDonald et al., 1980). The current levels of nitrate and nitrite used in various meat products as recommended by USDA (United States Department of Agriculture) Expert Panel in 1978 are given in Table 2. However, there are strong implications about reducing the use of nitrite in foods or banning it altogether, if alternative measures, equally effective against Clostridium botulinum, are commercially available (Leistner, 1979).

White (1976) has estimated the average daily ingestion of nitrate and nitrite for U.S. residents, and calculated the relative significance of various dietary sources. The data in Table 3 show that vegetables are the major source (86%) of nitrate in the average American diet; the rest originates from salivary excretion and cured meats (9.4%). The predominant portion of the ingested nitrite however, comes from saliva (76.8%) and a smaller but significant amount from cured meats (21%). More recently, Tannenbaum (1978) has shown that only about 3% of the nitrite ingested comes

Table 2. Nitrate and nitrite usage recommended by USDA Expert Panel.

Product	Levels of nitrate (mg/kg)	Levels of nitrite (mg/kg)
Cooked sausages	0	100-156
Fermented sausages	0	60-156
Dry cured cuts	300	100
Pickle cured products	0	110-200
Commercially-sterile products	0	50
Perishable-canned products	0	80-200
Shelf-stable products	0	156
Bacon[a]	0	120

[a]Level established by regulation on May 16, 1978.

Table 3. Relative significance of dietary sources of nitrate and nitrite: estimated daily ingestion for U.S. residents[a].

Source	Nitrate		Nitrite	
	mg	%	mg	%
Vegetables	86.1	86.3	0.20	1.8
Cured meat products	9.4	9.4	2.38	21.2
Bread	2.0	2.0	0.02	0.2
Fruits, juices	1.4	1.4	0.00	0.2
Water	0.7	0.7	0.00	0.0
Milk, milk products	0.2	0.2	0.00	0.0
Saliva	30.0[b]	--	8.62	76.8
Total	99.8	100.0	11.22	100.0

[a]Adapted from White (1976).

[b]Not included in total.

from the nitrite content in cured foods, about 15% from nitrite produced from nitrate in the mouth and about 82% from the nitrite produced in the human intestinal tract.

Various studies have shown that the nitrite level in saliva can increase markedly after consumption of meals containing nitrate-rich foods such as vegetables (Speigelhalder 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.

b. Amines in foods. As discussed earlier, N-nitrosamines can be formed from the N-nitrosation of secondary, tertiary and certain primary amines and quarternary ammonium compounds. Although the sources of nitrite in foods are well documented, information on the occurrence of the individual amines in foods is sparse.

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 amines 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 contents in butter and cheese were trace. Surprisingly, paprika, cayenne pepper and black pepper were found to contain fairly high levels of cyclic amines, PYR 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.

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 for cadaverine to 8.1 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 189 mg for putrescine per 100 g of 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. The volatile amines (methylamine, dimethylamine, trimethylamine, ethylamine, n-propylamine and isopropylamine) were detected in pork carcass meat used for Wiltshire bacon manufacture (Patterson and Mottram, 1974). The highest concentration detected was 1900 ug of methylamine per kg of fresh meat, which decreased during the curing process. Rice et al. (1976) have reported the presence of histamine, putrescine, tyramine, 2-phenylenthylamine in dry and semi-dry sausages. Various amines (dimethylamine, di-n-propylamine, PYR, morpholine and piperidine have been detected at 2 μg/kg or less in baked ham (Singer and Lijinsky, 1976). More recently, Nakamura et al. (1979) conducted a survey on the polyamines content of Japanese fresh and processed pork tissue. Slightly lower levels than those of Spinelli et al. (1974) were obtained. Aliphatic polyamines have also been isolated from soybeans (Wang, 1972), barley and wheat (Morruzzi and Cadaverg, 1964) and other plant tissues (Tabor and Tabor, 1964).

As with most compounds, amines in foods are formed via 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 conversion such as the enzymatic conversion of trimethylamine oxide to trimethylamine (Tar, 1940); (c) aldehyde amination as in the amination and transamination of aldehydes which is the potential pathway for most monoamines associated with foods (Hartmann, 1967; Maier, 1970); (d) phospholipid decomposition, for example, the formation of ethanolamine from the splitting of cephalin (Hrdlicka and Janicek, 1964), and (e) thermal amino decomposition which accounts for the appearance of a wide variety of amines, for example, ethanolamine, methylamine, propylamine and either iso- or pentylamine were found during heating of cysteine or cystine (Mulders, 1973). Velisek and Davidek (1974) also postulated that amines in foods could easily be formed during the non-enzymatic browning process.

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

N-Nitrosamines in Cured Meat Systems

Most of the published results on the occurrence of N-nitrosamines in cured meats have been summarized by Scanlan (1975) and Sen (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 percentage 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 DMN, N-nitrosodiethylamine (DEN), NPYR and N-nitrosopiperidine (NPIP).

a. Cured meats other than bacon. 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 DMN, NPYR and NPIP were sporadically found in frankfurters, 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). The reaction between nitrite and black pepper was reported to be

responsible for the formation of NPIP, whereas paprika predominantly produced NPYR. 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., 1981). 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 study conducted by Holland et al. (1981), the predominant N-nitrosamines detected were DMN and N-nitrosomorpholine (NMOR) and, generally, values of <4 μg/kg were obtained for each N-nitrosamine. However, these are presumptive N-nitrosamine levels since mass spectral confirmation of their identities was not achieved. It has been suggested that the detectable levels of NMOR in the Canadian study (Holland et al., 1981) were attributed in part to the use of morpholine as an anti-corrosion agent in the steam supply.

b. Bacon systems. To date, 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, DMN have been isolated consistently from cooked bacon (Table 4). 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 being 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 5). 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.

Periodic surveillance of NPYR in cooked bacon samples (Greenberg, 1976; Havery et al., 1977; Sen et al., 1977) suggested that progress has been made in decreasing the formation of NPYR in fried bacon over the years 1971-1976. This has been associated with improved control of processing procedures, reduced concentrations of nitrite and increased levels of ascorbate. In spite of these improvements, little further reduction has occurred in the majority of brands since then.

Table 4. N-Nitrosamine formation (μg/kg) in fried bacon[a].

Investigators	N-Nitrosopyrrolidine		N-Nitrosodimethylamine	
	Bacon	Cooked-out fat	Bacon	Cooked-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 5. Percentage of N-nitrosamines in the fumes produced during the frying of bacon[a].

Investigators	N-Nitrosamine (%)		
	NPYR	DMN	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.

c. Mechanism(s) of NPYR formation. The consistent occurrence of NPYR in fried bacon and cooked-out fat has led to an intensive search for both the precursors and mechanism(s) that could account for its formation. Although model system studies have implicated a number of compounds including PRO, collagen, putrescine, spermidine, PYR and glycyl-L-glycine as possible precursors of NPYR (Gray, 1976), the most probable precursor of NPYR in bacon appears to be PRO. Free PRO 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 PRO 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 2). One pathway involves the initial N-nitrosation of PRO, followed by decarboxylation, while in the other, PRO is first decarboxylated to PYR followed by N-nitrosation to NPYR. Since the conversion of NPRO to NPYR occurs at a much lower temperature than the transformation of PRO to PYR, the pathway involving the intermediacy of NPRO is thus the more likely route (Bharucha et al., 1979). It has been reported that preformed NPRO 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

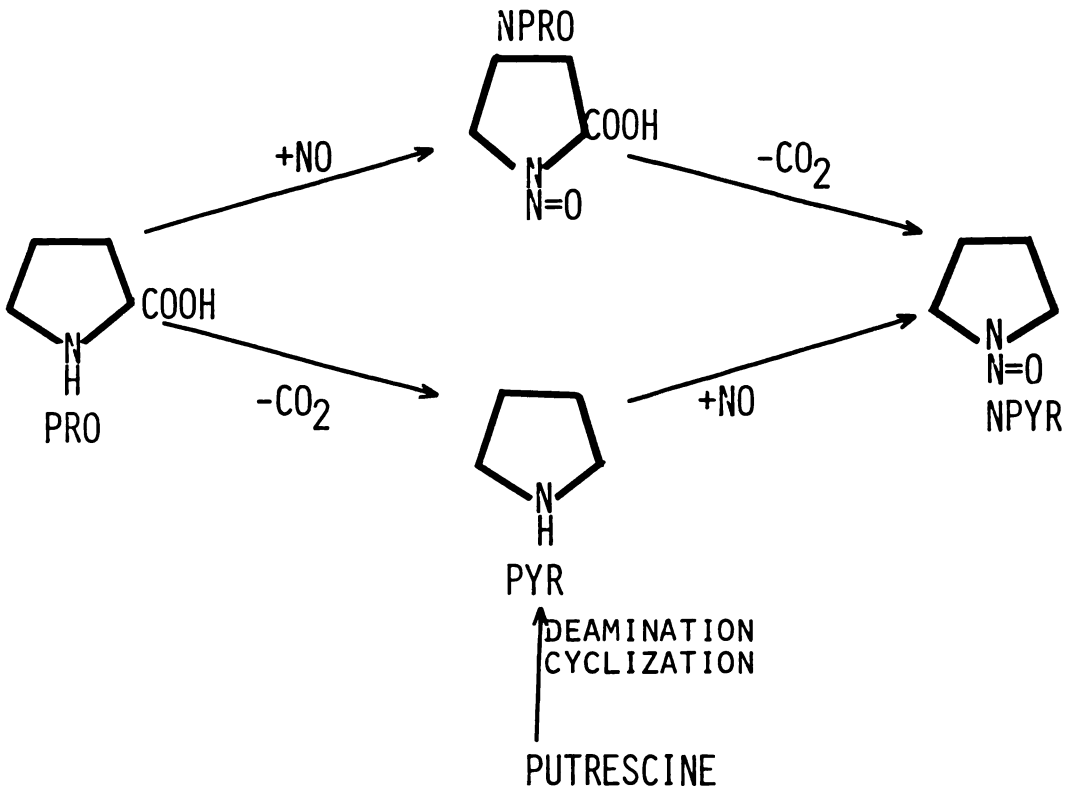


Figure 2. Possible pathways of N-nitrosopyrrolidine formation in bacon.

formation of NPYR (Sen et al., 1976a). However, this by no means rules out the intermediacy of NPRO which could be formed at the higher temperatures attained during the frying process (Bharucha et al., 1979).

The mechanism(s) of NPYR formation has been studied by Coleman (1978) and Bharucha et al. (1979). Coleman (1978) reported that the requirement of a high temperature, the inhibitory effects of water and antioxidants, and the catalytic effect 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 DMN 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· (Figure 3). Since NO·

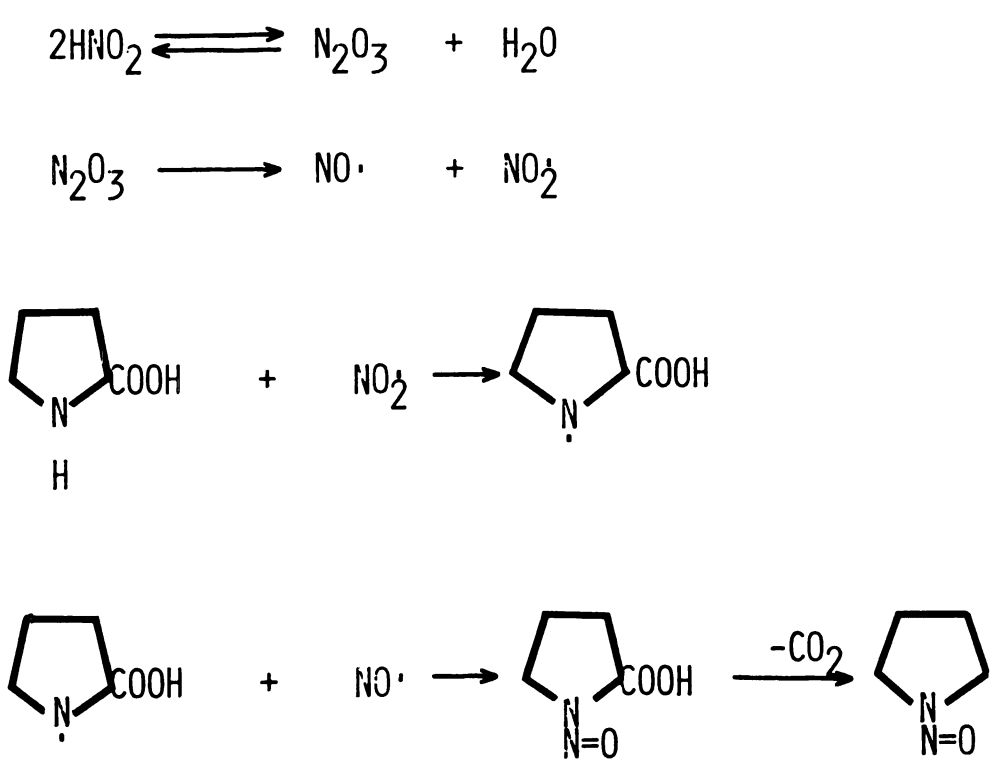


Figure 3. Free radical mechanism of N-nitrosopyrrolidine formation in bacon (Bharucha et al., 1979).

is relatively stable, it was 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).

d. 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 procedures, presence of lipophilic inhibitors, and possibly smoking.

d1. 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 (Bharucha et al., 1979). Bharucha et al. (1979) explained the reduced yields of N-nitrosamines during grilling as being due to the cooked-out fat running out of the 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°C, while samples from the same belly, fried to the same "doneness" at 204°C for 4 min, produced 17 μg/kg of NPYR. Bharucha et al. (1979) reported that the maximum amount of

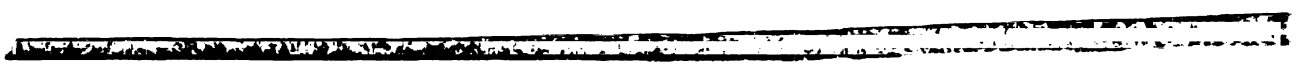
N-nitrosamine was produced when the bacon was fried for 12 min at 360°F, after starting with a cold frying pan. Very little N-nitrosamine was found in the rendered fat after 4 min of heating; however, the N-nitrosamine level increased sharply with time and reached a maximum at around 12 min and then began to decline. Two explanations were offered to explain the initial low formation of N-nitrosamine: (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.

d2. 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).

d3. N-Nitrosamine inhibitors. Ascorbic acid and its derivatives, and α-tocopherol have been widely studied as inhibitors of N-nitrosamine reactions in bacon (Greenberg, 1973; Sen et al., 1976a; Fiddler et al., 1978; Mergens and Newmark, 1979; Bharucha et al., 1980). The effect of sodium ascorbate on NPYR formation is variable, complete inhibition is not achieved, and although results indicate lower levels of NPYR in ascorbate-containing bacon, there are examples of increases (Mottram and Patterson, 1977). Recently, it has been concluded (Bharucha et al., 1979) that the essential but probably not the only requirements for a potential



anti-N-nitrosamine agent in bacon are its (a) ability to trap NO· radicals, (b) lipophilicity, (c) non-steam volatility and (d) heat stability up to 174°C (maximum frying temperature). These appear to be important requirements since the precursors of NPYR have been associated with bacon adipose tissue (Fiddler et al., 1974). Consequently, ascorbyl palmitate has been found to be more effective than sodium ascorbate in reducing N-nitrosamine formations (Pensabene et al., 1979b), while long chain acetals of ascorbic acid, when used at the 500 and 1,000 mg/kg levels have been reported to be capable of reducing the formation of N-nitrosamines in the cooked-out fat by 92 and 97%, respectively (Bharucha et al., 1980).

The inhibition of formation of NPYR and DMN in fried bacon by the use of cure-solubilized α-tocopherol (500 mg/kg) has been demonstrated by Fiddler et al. (1978). Walters et al. (1976) also reported reduced levels of N-nitrosamines in the vapors during the frying of bacon in fat containing α-tocopherol. It has been shown that α-tocopherol is dispersed 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). Controlled addition of this antioxidant may be an effective and practical way of reducing the concentration of N-nitrosamines in cooked bacon and minimizing the remote possibility of any health hazards arising from the

consumption of such foods (Sen, 1980).

d4. 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 had been either stored for 1 week in a refrigerator or frozen for 3 months and then thawed before using. It was suggested that the higher levels of NPYR results from the increase in both amines and amino acids that occurs during extended storage (Pensabene et al., 1980). Several investigators (Lakritz et al., 1976; Gray and Collins, 1977b) have shown that the free PRO contents in whole and lean tissue of green pork bellies increased approximately 50% after storage at $2^{o}C$ for 1 week. Over the same period, free PRO in the adipose tissue increased approximately 90%.

d5. 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 seem 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).

To date, the majority of 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 (Pensabene et al., 1979b; Nitrite Safety Council, 1980). NPYR levels ranging from 39-89 µg/kg were reported by Pensabene et al. (1979b), while those cited by the Nitrite Safety Council (1980) ranged from traces - 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 obviously are necessary (Nitrite Safety Council, 1980).

Toxicology of N-Nitroso Compounds

As mentioned earlier, the major concern of nitrite is the formation of carcinogenic N-nitroso compounds. Most N-nitrosamines have been shown to initiate tumors in at least one animal species, and all animal species which have so far been tested are susceptible to N-nitrosamine carcinogenesis (Preussmann et al., 1976). The carcinogenic potency of different N-nitroso compounds, however, varies a great

deal. N-Nitrosodialkylamines (e.g. DEN, DMN) are highly potent, whereas N-nitrosamines with branching and consequently fewer hydrogens at the α-carbon (e.g. N-nitrosodiethanolamine, 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).

EXPERIMENTAL

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

Materials

Reagents

All chemicals and solvents employed were of analytical grade and used without further purification. They were purchased from Mallinckrodt Inc. (Paris, KY) except where specified.

NPYR, PYR and L(-)PRO were purchased from Aldrich Chemical Co. (Milwaukee, WI). NPRO was synthesized from L(-)PRO according to the method of Hansen et al. (1974). The purity of NPRO was checked by elemental analysis (Spang Microanalytical Laboratory, Eagle Harbor, MI) and the results were as follows: found--41.61% C, 5.51% H, 19.42% N, 33.16% O; calculated-41.55% C, 5.35% H, 19.45% N, 33.32% O. Diazomethane was prepared from Aldrich N-methyl-N-nitroso-p-toluenesulfonamide as directed. Methylene chloride was

obtained from J.T. Baker Chemical Co. (Phillipsburg, N.J.), paraffin and silicone oil (Fisher Scientific Co., Fair Lawn, N.J.) and column packing materials from Supelco Co., Inc., Bellefonte, PA.

Meat samples

Three different brands of commercially available 1 lb packages of regular sliced bacon, and lard were purchased from local supermarkets. A 5 kg green pork belly (after 24 h slaughter) was obtained from the Meat Laboratory at Michigan State University (East Lansing, MI).

Apparatus

Two mL cryule^{R} glass ampules (Wheaton Scientific Co., Millville, N.J.), 2-dram and 6-dram screw cap glass vials (Kimble, Owens, IL) were used in the extraction steps instead of separatory funnels, 12 mL graduated conical centrifuge tubes and an assortment of glassware and syringes commonly used in laboratory work.

A Reacti-Therm^{TM} Heating Module (Pierce Chemical Co., Rockford, IL) and a Fisher Hi-Temp^{TM} bath model 160 (Fisher Scientific Co., Fair Lawn, N.J.) were used as oil wells and oil baths, respectively.

The gas chromatograph (GC) systems were comprised of a Hewlett-Packard Model 5830A with a flame ionization detector (FID) and a Hewlett-Packard Model 18850 Terminal. The GC columns were either 2 m x 2 mm i.d. or 2 m x 4 mm i.d. glass columns (Supelco Co., Inc., Bellefonte, PA).

Other essential apparatus included: a Corning PC-35 hotplate, a Fisher Mini-Shaker and a Fisher Ultra-Sonic Vibrator (Fisher Scientific Co., Fair Lawn, N.J.), a Sears Presto Telfon-coated electric skillet of dimension 28 x 35 cm (Sears and Roebuck, Chicago, IL), an Oster food grinder Model 945-08-H (Oster Corp., Milwaukee, WI), a RePP Sublimator (RePP Industries, Inc., Gardiner, N.Y.) and a thermocouple (Omega Engineering, Inc., Stamford, CT).

Methods

Preparation of bacon and green pork belly samples

All preparative steps were carried out in a cold storage room at $2^{o}C$. Bacon strips from different packages, but of the same brand, were randomized to give uniform samples. Some of these were further separated into adipose and lean tissues. Three 230 g samples of each of the randomized whole bacon, adipose and lean components were individually wrapped with aluminum foil and used immediately for the frying experiments. Approximately 50 g of each of aforementioned samples were ground twice in an Oster food grinder equipped with a 1/8 in. plate and thoroughly mixed prior to crude fat and moisture determinations.

Whole skinned green pork belly and its separated adipose and lean components were cut into small pieces and ground according to the procedure outlined above. Part of the homogenized samples were used for crude fat and moisture

determinations, while 300 g of each of the ground adipose and lean tissues were freeze-dried in a RePP Sublimator with a glycol setting of -15°C. The freeze-dried samples were stored under a nitrogen atmosphere in a closed-lid container at -20°C for wet model system studies.

Proximate analyses of bacon and green pork belly samples

a. Crude fat. Crude fat determinations were made by ether extraction with Goldfisch apparatus according to the A.O.A.C. procedure (1975; 24.005).

$$\% \text{ crude fat} = \frac{\text{weight of ether extract}}{\text{fresh sample weight}} \times 100$$

b. Moisture. Moisture determinations were carried out using the A.O.A.C. oven drying method (1975; 24.003).

$$\% \text{ moisture} = \frac{\text{fresh sample weight - dried sample weight}}{\text{fresh sample weight}} \times 100$$

Bacon frying procedures

a. Skillet calibration. The electric skillet was calibrated using a thermocouple according to the modified procedure of Owens and Kinast (1980). Approximately 320 g of lard were placed into the skillet and the thermostat was set at 171°C (340°F). The temperature was recorded every 30 seconds for 16 min while stirring the lard in a "figure 8" motion. A range of temperatures or the skillet temperature distribution profile was also recorded by placing the thermocouple at 16 different locations and recording the temperature

at each location for 2.6 min at 30 second intervals. The oil was poured off and the excess was removed with paper towels. The procedure was repeated at least twice daily if bacon was fried continuously during the day. The thermostat was adjusted to produce a similar range of skillet temperatures.

b. Bacon frying

b1. Hot skillet frying. The skillet was turned on and the temperature allowed to cycle at least 10 min while the thermostat was set at 171°C (340°F). As many strips of bacon as possible (approximately 230 g) were placed lengthwise in the skillet without overlapping but with room for turning. A fine thermocouple needle was inserted near the mid-points of the two center strips (making sure not to expose any part of it to the surface of the skillet) and the temperatures were recorded at 30 second intervals. The bacon was fried on each side for 3 min, and the remaining cooked-out fat was discarded. Lukewarm, mild soapy water was used to clean the skillet in between frying different brands.

b2. Cold skillet frying. The procedure was similar to (b1) above except that the skillet received no preheating and the bacon strips were fried for 4 min on either side.

The separated adipose and lean components were fried according to the hot skillet frying procedure.

Heating conditions for model system studies

All model system studies were conducted in oil wells at pre-set temperatures (80-160°C at 20°C intervals) for 2, 4, 6, 8, 10 and 12 min, using a Reacti-Therm™ heating module. Small oil wells were made by filling the holes of the heating block with 7 mL of paraffin oil. The oil was replenished whenever necessary during the heating process. A thermometer was placed in one of the oil wells to monitor the temperature. For temperatures above 160°C, a Fisher Hi-Temp™ bath filled with silicone oil was used.

Decarboxylation reactions in dry model systems

a. Thermal degradation of NPRO to NPYR. 0.1 mMol of NPRO was carefully weighed and sealed inside 2 mL glass ampules and heated for specific periods of time over a wide range of temperatures as described. After heating, the ampules were cooled and analyzed immediately, or stored in a freezer at -20°C. The samples were extracted with 3 x 1 mL aliquots of methylene chloride, stirring with a mini-shaker for 1 min for each extraction. All ampules were extracted again with 2 x 0.5 mL aliquots of methylene chloride using an Ultra-Sonic Vibrator to achieve complete solution of the ampule contents. The ampules were subsequently rinsed with 2 x 1 mL aliquots of the same solvent. The combined methylene chloride extracts (approximately 6 mL) were washed with 2 x 3 mL aliquots of deionized water to remove traces of unreacted NPRO. The purified extract was dried over anhydrous sodium

sulfate and made up to a 10 mL total volume for GC analysis.

Unheated ampules of NPRO were analyzed in a similar manner and used as a control to calculate percent decomposition. Recoveries of 50, 100 and 150 μg of NPYR added to the unheated vials of NPRO varied from 85-88% with an average of 86 ± 2% (mean ± standard deviations).

b. Thermal degradation of L(-)PRO to PYR. A similar set of ampules was prepared by replacing NPRO with 0.5 mMol of L(-)PRO. The ampules were heated over the temperature range 80-200°C at 20°C intervals, and at 210 and 230°C for specified periods of time. The contents of each ampule were dissolved in 1 mL methylene chloride, and then analyzed by GC. Some samples were agitated using an Ultra-Sonic Vibrator to facilitate the solution process. The contents of unheated ampules were extracted and analyzed in a similar manner.

N-Nitrosation and decarboxylation reactions in wet model systems

The N-nitrosation reactions of L(-)PRO and PYR with sodium nitrite ($NaNO_2$), and the decarboxylation reactions of L(-)PRO and NPRO were investigated under conditions simulating the frying of bacon in wet model systems as described below.

a. Preparation of chemical solutions. The chemical solutions used in wet model system studies were made up to the desired concentrations as shown in Table 6. The $NaNO_2$

Table 6. Chemical solutions used in wet model system studies.

Chemicals	Molecular weight (g)	Concentration in deionized water	
		g/10 mL	mMol/uL
L(-)Proline	115.73	2.8783	0.05/20
N-Nitrosoproline	144.13	1.8016	0.05/40
Pyrrolidine	71.72	1.7930	0.05/20
Sodium nitrite	69.00	1.7250	0.05/20

and PYR solutions were prepared just prior to each assay.

b. Assay procedures

b1. Preparation of wet model systems. The compositions of five different wet model systems are tabulated in Table 7. A set of sealed ampules containing the reaction

Table 7. Composition of wet model systems.

Type of pork tissue[a]	Weight of pork tissue (g)	Deionized water (uL)	Chemical solutions (uL)			
			L(-)PRO	NPRO	PYR	$NaNO_2$
Adipose[b]	0.16	--	20	--	--	20
Lean[c]	0.08	80	20	--	--	20
Adipose	0.16	--	--	--	20	20
Adipose	0.16	--	--	40	--	--
Adipose	0.16	20	20	--	--	--

[a]Freeze-dried pork tissue from green pork belly.

[b]Original composition of adipose: 80% dry matter, 20% moisture.

[c]Original composition of lean: 40% dry matter, 60% moisture.

mixture was prepared for each model system study and incubated in oil wells as previously described.

Samples were prepared by careful delivery of freeze-dried pork tissue and reactants (in the order from left to right, Table 7) into the ampules, sealed, mixed thoroughly and held at 2°C for 2 h before heating. Water, in volumes of chemical solution(s) and deionized water, was added to rehydrate the freeze-dried pork tissue. The volume of chemical solutions introduced was required to give a 0.05 mMol of the reactant. Due to the volatile nature of PYR, this solution was added to ampules chilled with dry ice. After heating, the ampules were quickly cooled and analyzed immediately or stored at -20°C.

A set of nitrite blanks containing 0.05 mMol of $NaNO_2$, in the absence of other reactants were prepared by adding the appropriate amounts of deionized water to freeze-dried pork tissue. These were heated at each reaction temperature: 80, 120 and 160°C, for 6 min and analyzed in a similar manner.

b2. Isolation and preparation of reaction products for GC analysis. For control experiments and to check for artifact formation, unheated ampules containing the appropriate amounts of freeze-dried pork tissue and reactant(s) were analyzed simultaneously for each procedure.

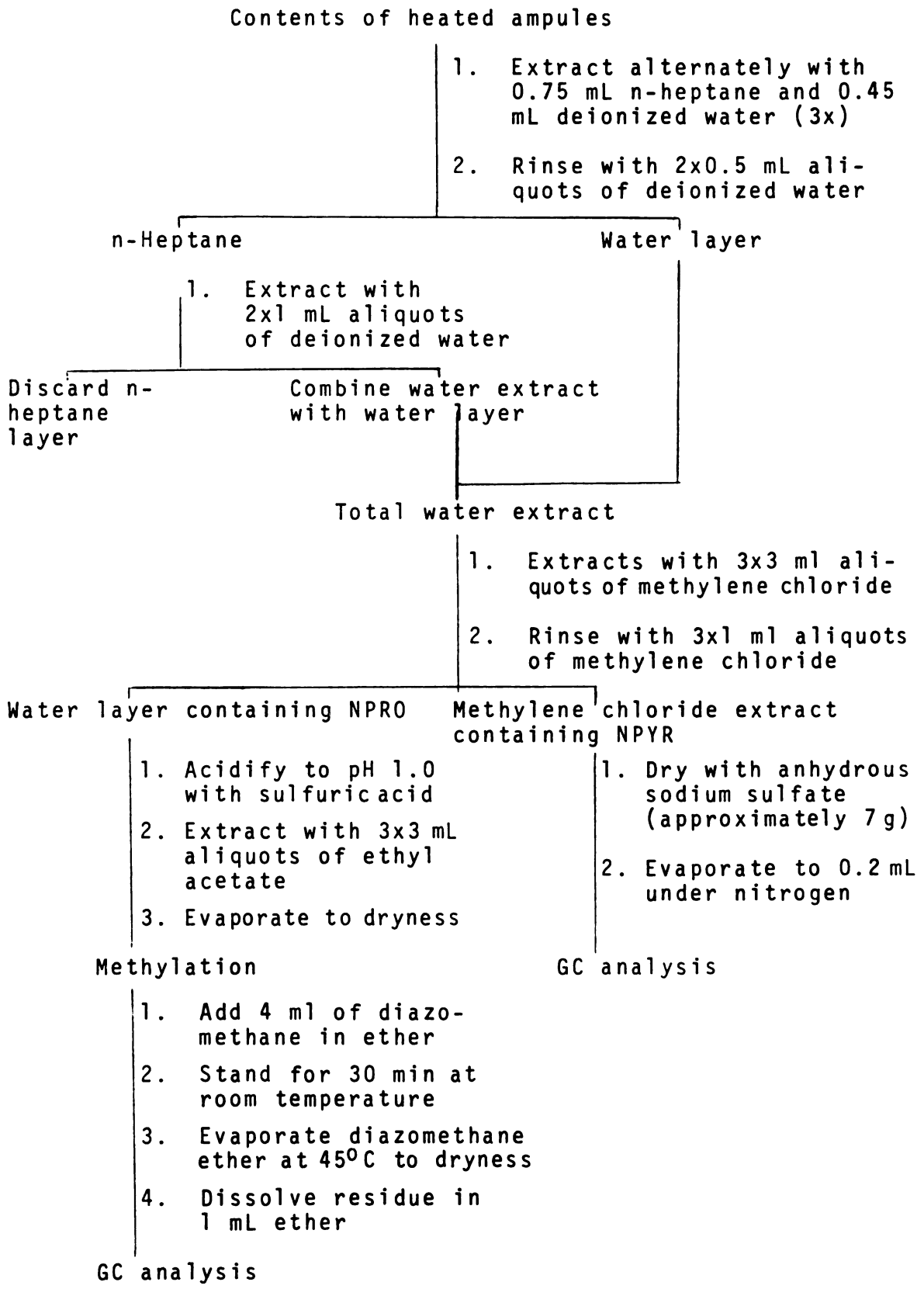
1) NPYR and NPRO analysis. The reaction products, NPYR and NPRO from heated adipose and NPYR from heated lean model systems containing 0.05 mMol each of L(-)PRO and $NaNO_2$ were extracted and concentrated in graduated conical centrifuge tubes as described in Figure 4. The total extraction time was 20-22 min; each extraction step was facilitated using a mini-shaker. Recoveries of NPYR (25, 50 and 75 µg) and NPRO (500, 1,000 and 1,500 µg) added to unheated adipose tissue and analyzed in a similar manner were 51 ± 2% and 37 ± 4%, respectively. Recovery of NPYR added to unheated lean tissue was 45 ± 2%.

NPYR, the N-nitrosation product of a heated wet model system containing adipose tissue, PYR and $NaNO_2$ (0.05 mMol of each reactant) was also extracted according to the procedure described above. To evaluate the decarboxylation reaction of 0.05 mMol of NPRO in wet model systems as a function of heating times and temperatures, the decomposition product, NPYR, was dissolved directly in 0.5 mL methylene chloride for GC analysis. Recovery of added NPYR (25, 50 and 75 µg) was 98 ± 1%.

2) PYR analysis. After decarboxylation of L(-)PRO (0.05 mMol) to PYR in a heated adipose system, the reaction mixture was dissolved in 0.4 mL of n-heptane. The organic phase was extracted with deionized water (2 x 0.4 mL) while stirring on a mini-shaker. The combined aqueous layers were filtered through glass wool. The residue was rinsed

Figure 4. Isolation and preparation procedure for NPYR and NPRO from wet model systems (modification of procedure of Bharucha et al. (1979)).

Note: saturated sodium chloride solution was used instead of deionized water for the extraction of NPYR from lean model systems.


Contents of heated ampules
1. Extract alternately with 0.75 mL n-heptane and 0.45 mL deionized water (3x)
2. Rinse with 2x0.5 mL aliquots of deionized water
n-Heptane
Water layer
1. Extract with 2x1 mL aliquots of deionized water
Discard n-heptane layer
Combine water extract with water layer
Total water extract
1. Extracts with 3x3 ml aliquots of methylene chloride
2. Rinse with 3x1 ml aliquots of methylene chloride
Water layer containing NPRO
Methylene chloride extract containing NPYR
1. Acidify to pH 1.0 with sulfuric acid
2. Extract with 3x3 mL aliquots of ethyl acetate
3. Evaporate to dryness
1. Dry with anhydrous sodium sulfate (approximately 7 g)
2. Evaporate to 0.2 mL under nitrogen
Methylation
GC analysis
1. Add 4 ml of diazomethane in ether
2. Stand for 30 min at room temperature
3. Evaporate diazomethane ether at 45°C to dryness
4. Dissolve residue in 1 mL ether
GC analysis

with deionized water and the combined filtrate and rinsings were made up to a final volume of 1 mL. Prior to GC analysis, the aqueous solution was filtered through a membrane filter (Metricel^{R} Membrane Filter, 0.45 μm) to remove any minute particles which may interfere with the analysis. Recovery of added PYR (25 and 50 μg) was 94 ± 2%.

GC analysis

The quantitative and/or qualitative determination of NPYR, PYR and NPRO methyl ester were conducted using a Hewlett-Packard Model 5830A gas chromatograph equipped with a Hewlett-Packard 18850 Terminal (Table 8). The signal response from FID was integrated as peak area. Linearity of response was established using standard solutions of varying concentrations. Standard curves were prepared for calculating the concentrations of each reaction product in the samples. All samples were determined in duplicate except for blanks and recoveries where triplicate analyses were carried out. At least two injections were made for each GC analysis.

Calculations

The analyses of variance for the bacon frying experiments were carried out according to the method of Cochran and Cox (1950). Percent yields of reaction products were expressed as

$$\frac{\text{mMol product}}{\text{mMol reactant}} \times 100\%.$$

Table 8. Gas chromatographic conditions used in the determinations of N-nitrosopyrrolidine, pyrrolidine and N-nitrosoproline methyl ester.

	NPYR	PYR	NPRO methyl ester
Glass column and its packing material	2 m x 4 mm i.d. 10% Carbowax 20M-5% KOH on 80/100 Chromosorb W AW	2 m x 2 mm i.d. 80/100 Chromosorb 103 or (2 m x 4 mm i.d. 3% SP-2401-DB on 100/120 Supelcoport)[b]	2 m x 4 mm i.d. 10% OV-101 on 80/100 Supelcoport
Injection temperature	200°C (140°C)[a]	160°C (110°C)[b]	160°C
Column temperature	180°C (140°C)[a]	140°C (90°C)[b]	140°C
FID temperature	300°C (300°C)[a]	300°C (250°C)[b]	300°C

[a]Alternate conditions for quantitating NPYR, decomposition product of NPRO in adipose systems.

[b]GC conditions for qualitative determination of PYR in dry model systems.

The first order rate constants for the decarboxylation reactions of L(-)PRO and NPRO were calculated according to the rate equation (Fan and Tannenbaum, 1972):

$$\text{rate} = k\ (\text{reactant})$$

The pH-dependent rate constants for N-nitrosation reactions were calculated on the basis of the proposed rate equation by Mirvish (1975):

$$\text{rate} = k\ (\text{amine})(\text{nitrite})^2$$

The rate of formation of NPRO from L(-)PRO and $NaNO_2$ in adipose systems was estimated as:

$$\text{rate} = k_1\ (\text{amine})(\text{nitrite})^2 - k_2\ (\text{NPRO})$$

where k_1 was the rate of formation of NPRO and k_2 was the rate of decomposition of NPRO.

The fitting of equations to data for the estimation of rate constants was carried out using the least square criterion known as KINFIT 4. This program was first described by Dye and Nicely (1971).

RESULTS AND DISCUSSION

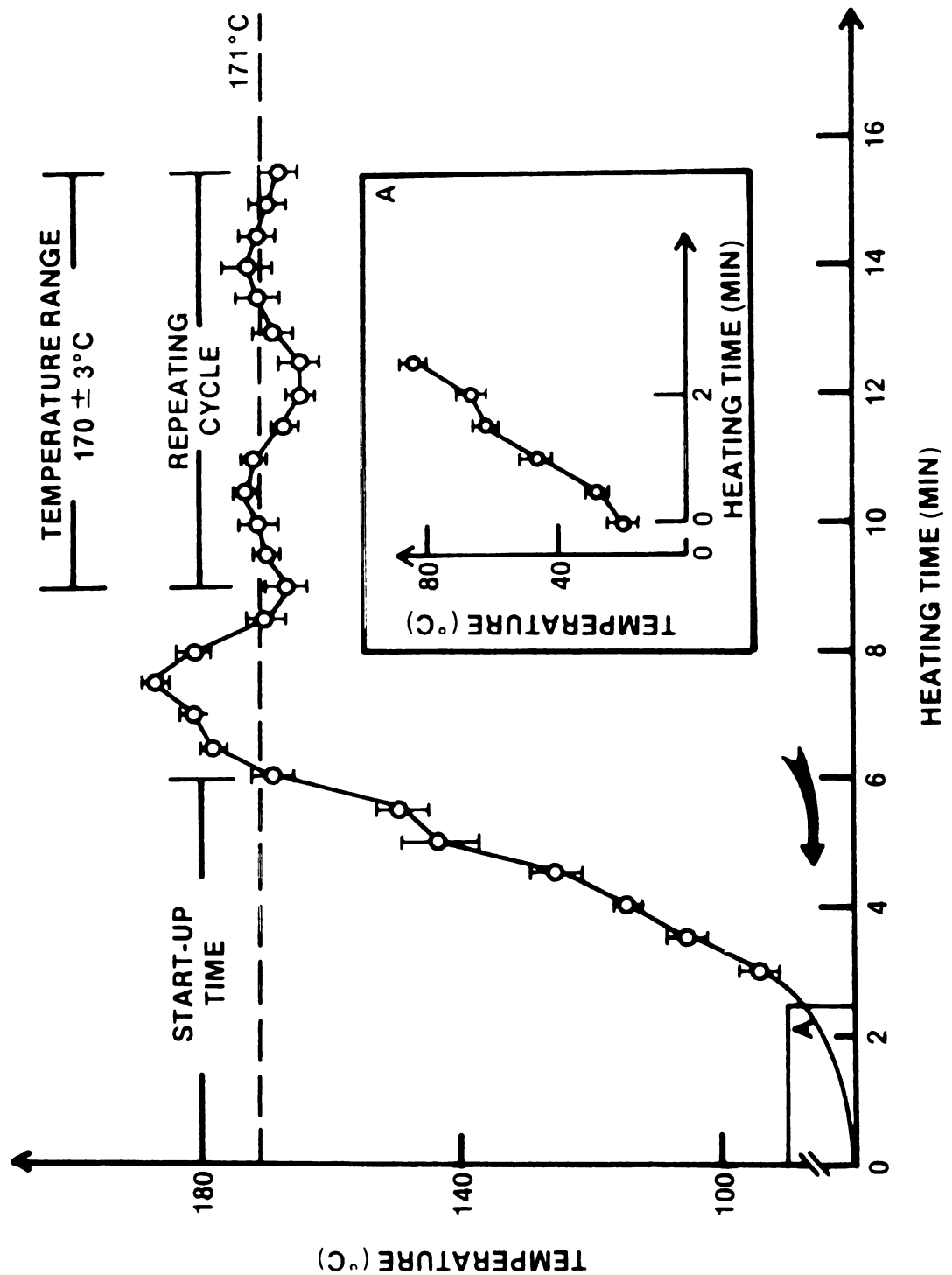
Preliminary Experiments With Bacon Frying

It is well established that NPYR formation in bacon is markedly temperature dependent (Gray and Randall, 1979). Therefore, as a prelude to kinetic studies on its formation, the internal temperatures of bacon attained during the frying process were established. The two different methods employed were hot and cold skillet frying, which resembled the conventional practices of food service- and home-preparation of fried bacon (Wasserman et al., 1978).

Skillet calibration

To better understand the skillet temperature profile, the fluctuation of skillet temperature as a function of heating time was investigated with the thermostat set at 171°C (340°F) (Figure 5). After the skillet was turned on, there was a 6 min delay (start-up-time) before the set value was reached. Following that, the temperature of the skillet reached a maximum of 187-189°C, exceeding the thermostat set point by approximately 16°C. The skillet temperature then assumed a cycling pattern with an average temperature range of 170 ± 3°C. Hence, for hot skillet frying, the skillet was turned on for 10 min before the actual frying procedure was carried out.

Figure 5. Skillet heating, start-up and cycling.


TEMPERATURE RANGE
170 ± 3°C
REPEATING CYCLE
171°C
START-UP TIME
TEMPERATURE (°C)
180
140
100
0
2
4
6
8
10
12
14
16
HEATING TIME (MIN)
A
TEMPERATURE (°C)
80
40
0
0
2
HEATING TIME (MIN)

The temperature distribution in the skillet was also measured, after the skillet temperature had assumed a cycling pattern with thermostat at 171°C. A temperature spread of 17 ± 4°C was observed. Four isothermal areas of the conventional electric skillet could be identified as shown in Figure 6. These were:

Zone I --temperature around 155 ± 4°C, about 2 cm away from the edge,

Zone II --temperature around 172 ± 4°C, about 5.5 cm away from the edge, where the heating element was located.

Zone III--temperature around 165 ± 3°C, about 8.5 cm away from the edge, and

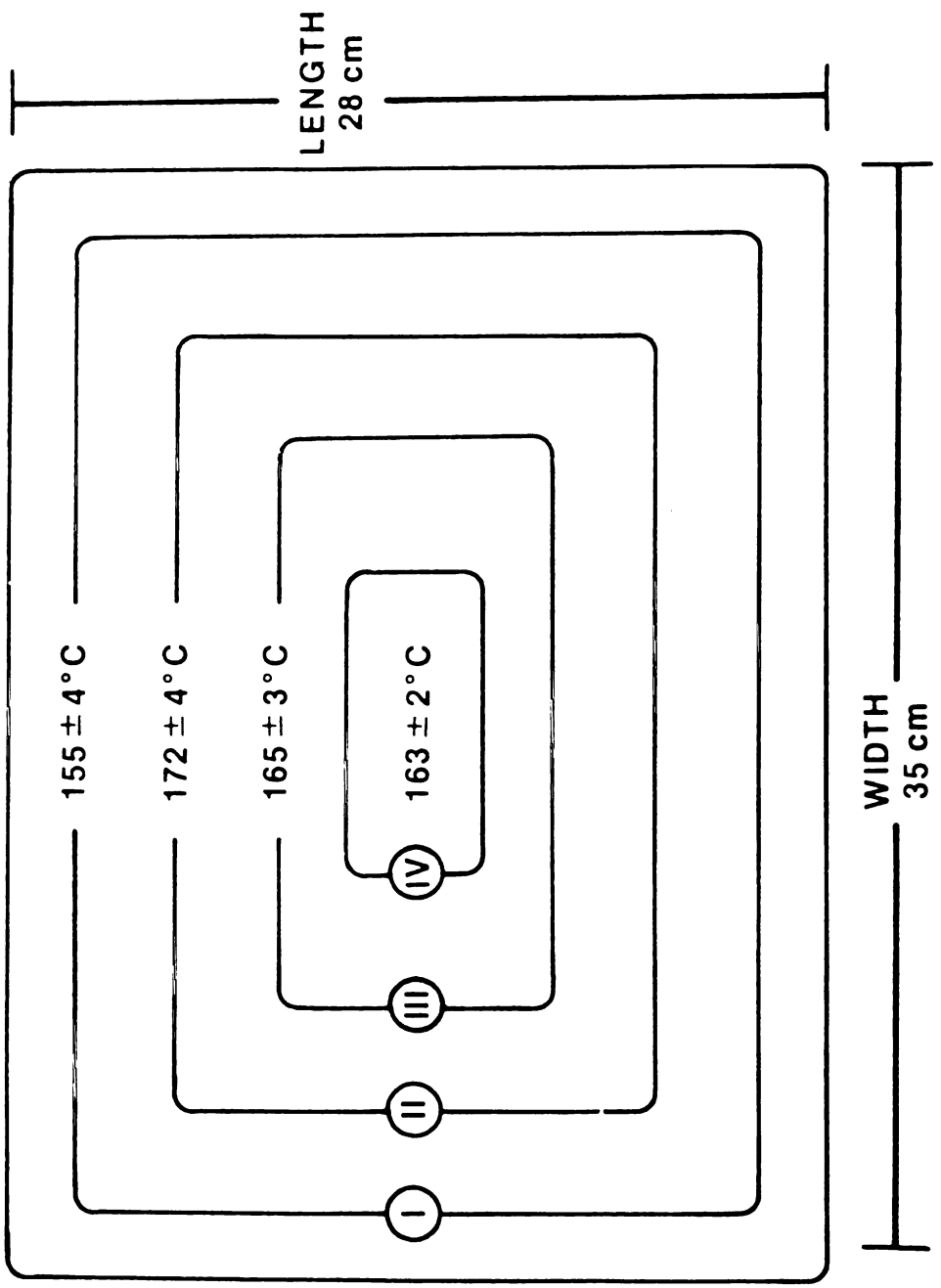
Zone IV --temperature around 163 ± 2°C, center region of skillet, about 12 cm away from the edge.

These results clearly indicate that the temperature over the entire cooking surface of the skillet was not uniform. This is an important consideration in evaluating bacon temperatures.

Hot and cold skillet frying

Three brands of commercial bacon strips were used in these frying procedures. These strips were approximately 2.5-3.0 mm thick and 22-25 cm in length, and had adipose to lean ratios of 2.19 ± 0.5 (mean and standard deviations). The relative distribution by weight of adipose and lean tissues in these samples were 68.7 ± 5.0 and 31.5 ± 5.0%,

Figure 6. Temperature profile of skillet set at 171°C (340°F)


LENGTH
28 cm
WIDTH
35 cm
155 ± 4°C
172 ± 4°C
165 ± 3°C
163 ± 2°C
I
II
III
IV

respectively. Crude fat and moisture contents of the bacon samples are tabulated in Table 9. The two variables were

Table 9. Mean values of crude fat and moisture contents of commercial bacon samples.

Sample	% Crude fat	% Moisture	% Total[a]
Whole bacon	64.1 ± 4.9[b]	21.1 ± 3.2	86.3 ± 1.7
Lean tissue	38.1 ± 2.2	43.5 ± 2.8	81.5 ± 0.2
Adipose tissue	81.3 ± 1.6	14.1 ± 1.1	95.4 ± 0.2

[a]% crude fat + % moisture.

[b]Mean and standard deviations (n=9).

inversely related to one another. Gray and Collins (1978) reported average values of 51.0 and 49.0% for adipose and lean, respectively for four commercially available bacon samples. The average moisture contents reported were 39.1% for whole bacon, 67.5% for lean and 11.9% for adipose. Pensabene et al. (1969b) reported a range of values of 42.4-58.5% for crude fat and 31.1-42.9% for moisture contents in their bacon samples. In comparison, the bacon samples used in this study had a slightly higher fat content. However, Schroder and Rust (1974) reported average fat contents ranging from 30 to 70% and concluded that there was as much variation within the same belly as among different bellies.

Figure 7 illustrates the internal temperatures reached during the frying of whole bacon using hot and cold skillets. The bacon temperature increased sharply with time during the hot skillet frying procedure, reaching 103 ± 8°C within 1 min and 164 ± 3°C at the end of the 6 min cooking period. However, a more gradual increase in bacon temperatures was observed using a cold skillet. Temperatures above 100°C were reached after approximately 3.5 min of frying. At the end of the 6 and 8 min frying periods, the temperatures recorded were 127 ± 5°C and 165 ± 6°C, respectively. A longer frying period, approximately 7 min, was required for the bacon to reach the same degree of "doneness" as those fried using the hot skillet. However, the corresponding final temperature was about 20°C lower.

The effect of compositional factors such as fat and moisture contents on the formation of NPYR in bacon have been discussed by several workers (Fiddler et al., 1974; Mottram et al., 1977; Gray and Collins, 1977b; 1978; Pensabene et al., 1979b). In this study, the correlation between compositional factors (fat, moisture) and bacon temperatures reached during frying in a preheated (hot skillet was investigated (Figure 8). Separated adipose and lean components from whole bacon samples (Table 9) were used. After the normal frying time of 6 min, it was observed that the lean bacon strips rarely exceeded 145°C while the adipose approached 165°C. Similar observations were made by Mottram

Figure 7. Internal temperatures reached during frying of whole bacon strips with preheated (hot) and cold skillets.

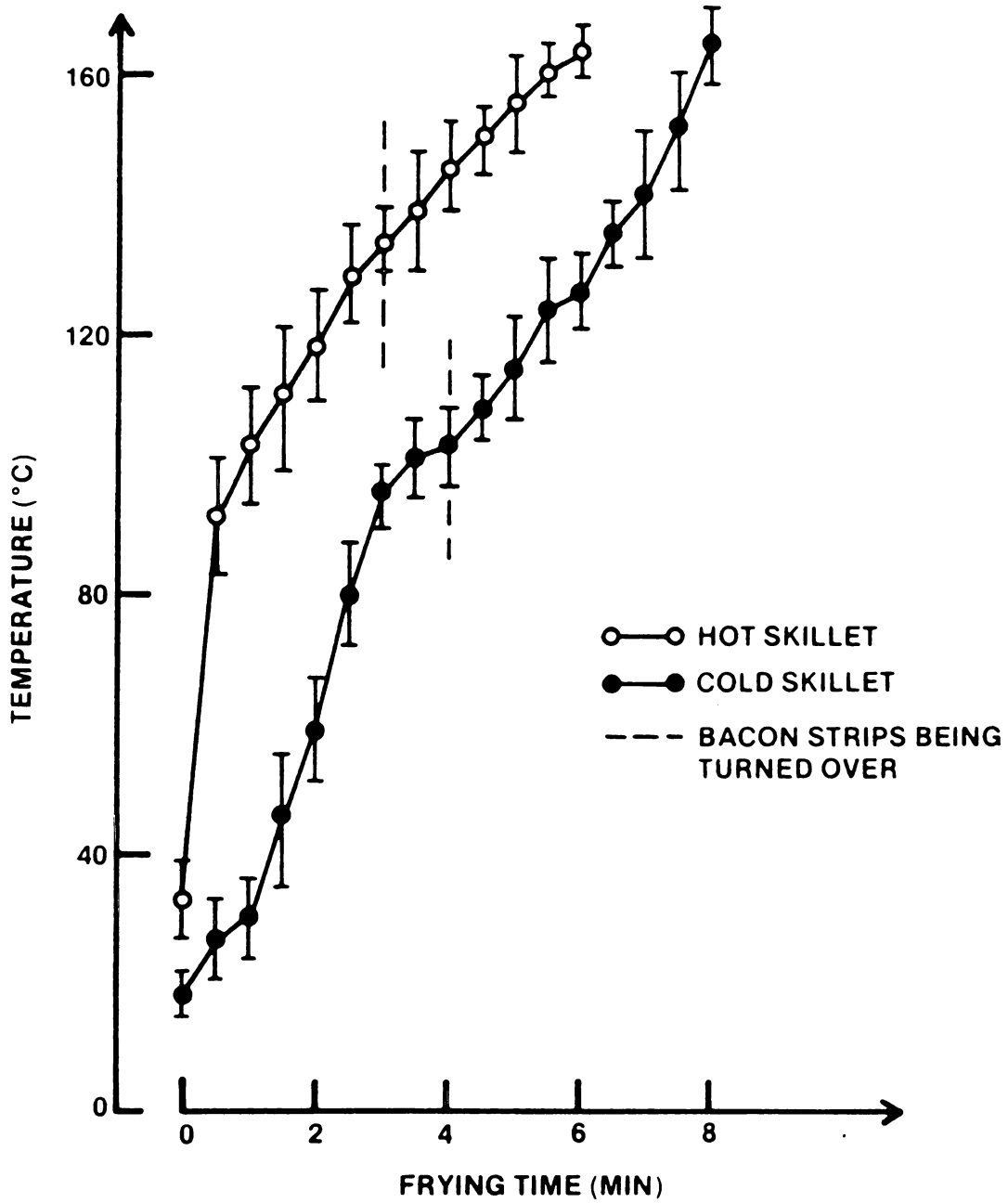
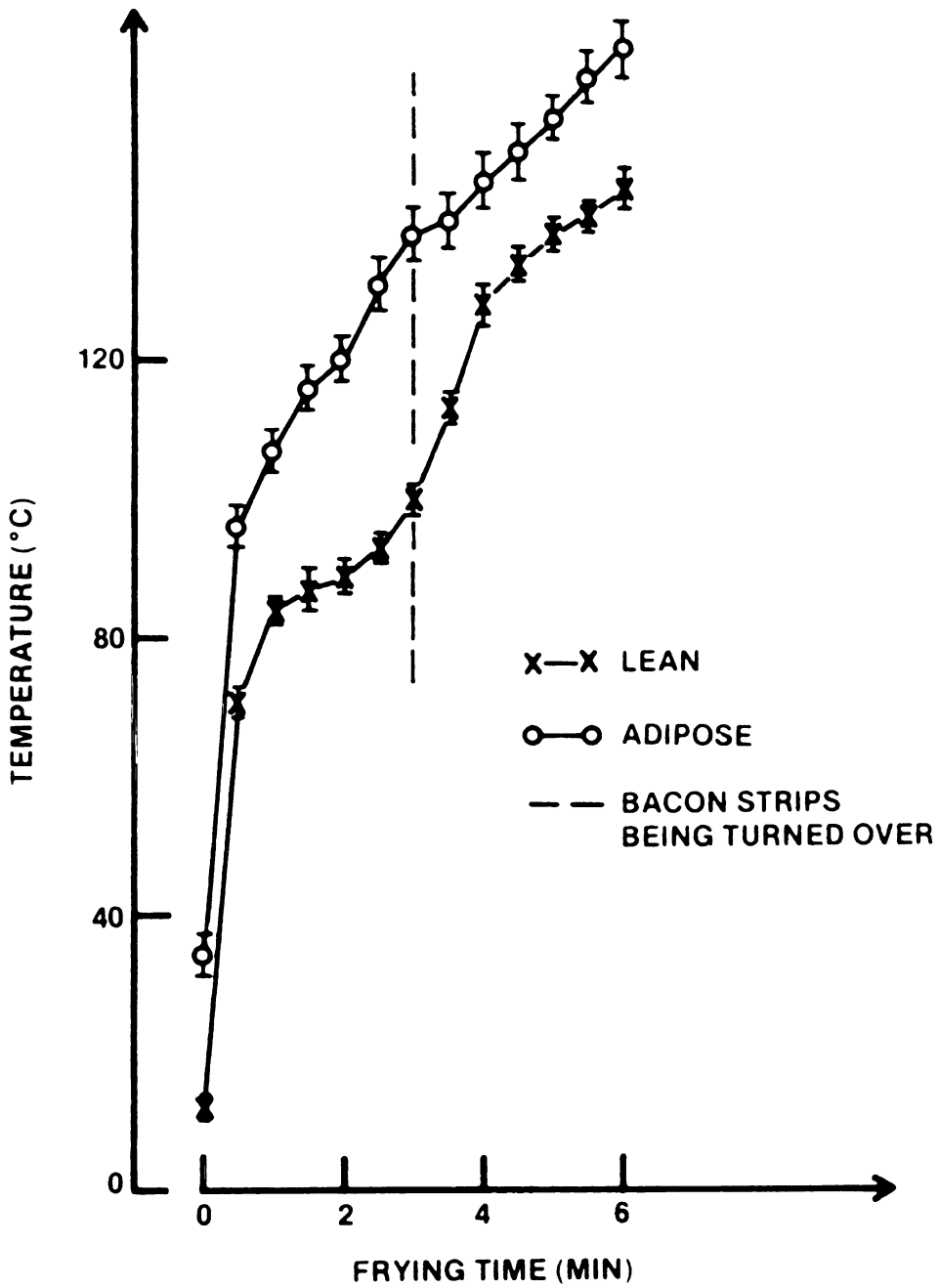

160
120
80
40
0
TEMPERATURE (°C)
0
2
4
6
8
FRYING TIME (MIN)
HOT SKILLET
COLD SKILLET
BACON STRIPS BEING TURNED OVER

Figure 8. Internal temperatures reached during frying of separated bacon components (adipose and lean) with a preheated (hot) skillet.


TEMPERATURE (°C)
120
80
40
0
0
2
4
6
FRYING TIME (MIN)
X—X LEAN
O—O ADIPOSE
— — BACON STRIPS
BEING TURNED OVER

et al. (1977) and Coleman (1978). The normal frying time employed by Mottram and his co-workers (1977) was 12 min at 178 ± 3°C. They reported a maximum lean temperature of 145°C and a slightly higher (150°C) adipose temperature. Coleman (1978), with the use of an electric cooker operating at its maximum setting reported values of 100°C and 200°C for the separated lean and adipose tissues, respectively. Total frying time was 6 min. As explained by other investigators (Fazio et al., 1973; Scanlan, 1975; Gray, 1976), the lower water content and low specific heat of fat generally results in more rapid heating of the adipose than the lean.

Results of the analyses of variance for the bacon frying experiments are given in Table 10. The F-ratio for

Table 10. Comparison of treatments sum of squares in bacon frying experiments.

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F-ratio
Treatments	3	19967.7	6655.9	5.01*
Hot vs Cold skillet	1	15084.3	15084.3	11.35**
Adipose vs Lean	1	3913.9	3913.9	2.95***
Whole vs Separated	1	969.5	969.5	0.73
Error	48	63802.2	1329.2	--

* Denotes significance at $p \leq 0.05$ level.

** Denotes significance at $p \leq 0.01$ level.

***Denotes significance at $p \leq 0.10$ level.

the main effects of different treatments (hot and cold skillets; varying compositional factors) was significant at $p \leq 0.05$ level. Bacon, fried with a hot skillet reached significantly ($p \leq 0.01$) higher internal temperatures than those fried with a cold skillet. The between-compositional variation was less significant. At the $p \leq 0.10$ level, the temperature variation was significant between adipose and lean and not significant between whole bacon and its separated components as a group. Further analysis of variance indicated that, while no significant difference in temperature was detected between adipose and whole bacon, there existed a significant difference between whole bacon and lean at the $p \leq 0.10$ level.

The large variations in temperatures reached at specific time intervals (Figures 7 and 8) during the frying of bacon samples could be explained by (a) uncontrolled sample variations which were more pronounced in whole bacon than in its separated adipose and lean components, and (b) effects of skillet temperature cycling and distribution as mentioned previously. In addition, bacon samples tend to corrugate during the frying process and thus may be contributory to temperature variation.

As mentioned earlier, temperature has a profound influence on NPYR formation. The range of temperatures employed in any studies of the mechanism of NPYR formation in bacon should be closely correlated with those reached in fried

bacon. In view of the results obtained in these preliminary studies, a temperature range of 80-160°C at a stepwise increment of 20°C was used for subsequent model system studies.

Model System Studies

A series of model system studies was initiated to examine the mechanism of NPYR formation in bacon. The reactants or the reaction mixtures employed in these systems were sealed inside 2 mL glass ampules which were then subsequently heated at specified temperatures. The systems were heated for 2, 4, 6, 8, 10 and 12 min at each designated temperature (80-160°C at 20°C intervals).

Decarboxylation reactions of NPRO and PRO in dry model systems

The thermal stabilities of 0.1 mMol NPRO and 0.5 mMol PRO in their crystalline state were examined in dry model systems.

Results of the decarboxylation of NPRO to NPYR as a function of time and temperature are presented in Table 11. No NPYR was detected at 80°C. However, decarboxylation of NPRO was initiated at 100°C with detectable levels of NPYR being produced after 8 min of heating. Thereafter, the formation of NPYR increased with increasing time and temperature. The maximum yield of NPYR was 2.46% at 160°C after heating the system for 12 min. In comparison, PRO was more stable than NPRO toward thermal stress (Table 12). PYR, the

Table 11. Percent thermal decarboxylation of N-nitrosoproline (0.1 mMol) to N-nitrosopyrrolidine in a dry model system.

Heating temperature (°C)	Heating time (min)					
	2	4	6	8	10	12
80	N.D.[a]	N.D.	N.D.	N.D.	N.D.	N.D.
100	tr[b]	tr	tr	0.06[c,d]	0.08	0.11
120	0.20	0.51	0.85	1.13	1.46	1.51
140	1.12	1.47	1.63	1.78	1.88	1.96
160	1.70	2.16	2.22	2.31	2.38	2.46

[a]None detectable; detection limit: 20 ng.

[b]Trace.

[c]% yield corrected for recovery.

[d]% standard deviations: ± 0.00 to 0.20 (n=2).

Table 12. Percent thermal decarboxylation of proline (0.5 mMol) to pyrrolidine in a dry model system.

Heating temperature (°C)	Heating time (min)					
	2	4	6	8	10	12
80 to 200	N.D.[a]	N.D.	N.D.	N.D.	N.D.	N.D.
210 ± 2	N.D.	tr[b]	tr	tr	tr	tr
230 ± 2	+[c]	+	+	+	+	+

[a]None detectable.

[b]Trace.

[c]Detectable amount of PYR.

decarboxylated product of PRO, was not detectable at temperature below 210°C. Its formation was observed at the end of a 4 min heating period at 210 ± 2°C. No attempt was made to quantitate the reaction product as PRO decarboxylated at a much higher temperature than would normally be encountered during frying of bacon.

A point worthy of mentioning in the extraction of NPYR was the removal of unreacted NPRO with deionized water from the methylene chloride extract prior to GC analysis. This is necessary since NPRO, if present, would readily decompose to NPYR under the GC analytical conditions employed for NPYR analysis. Deionized water is a preferred choice as the partition coefficient of NPRO between water and methylene chloride is 31 (Lijinsky et al., 1970). The reliability of the extraction procedure was tested in a control experiment containing 0.1 mMol NPRO in unheated ampules. No NPYR could be detected.

The gas chromatograms of the decomposition products of NPRO and PRO in dry model systems are shown in Figures 9 and 10, respectively. In addition to the reaction products of interest, there were several unidentified peaks associated with the decomposition reactions. Fan and Tannenbaum (1972) and Coleman (1978) have successfully demonstrated with model systems, that if decarboxylation does occur, loss of the N-nitroso group or denitrosation may occur simultaneously. Coleman (1978) augmented this argument by isolating PRO and

Figure 9. Gas chromatogram of N-nitrosopyrrolidine formed by heating N-nitrosoproline (0.1 mMol) in dry model systems.

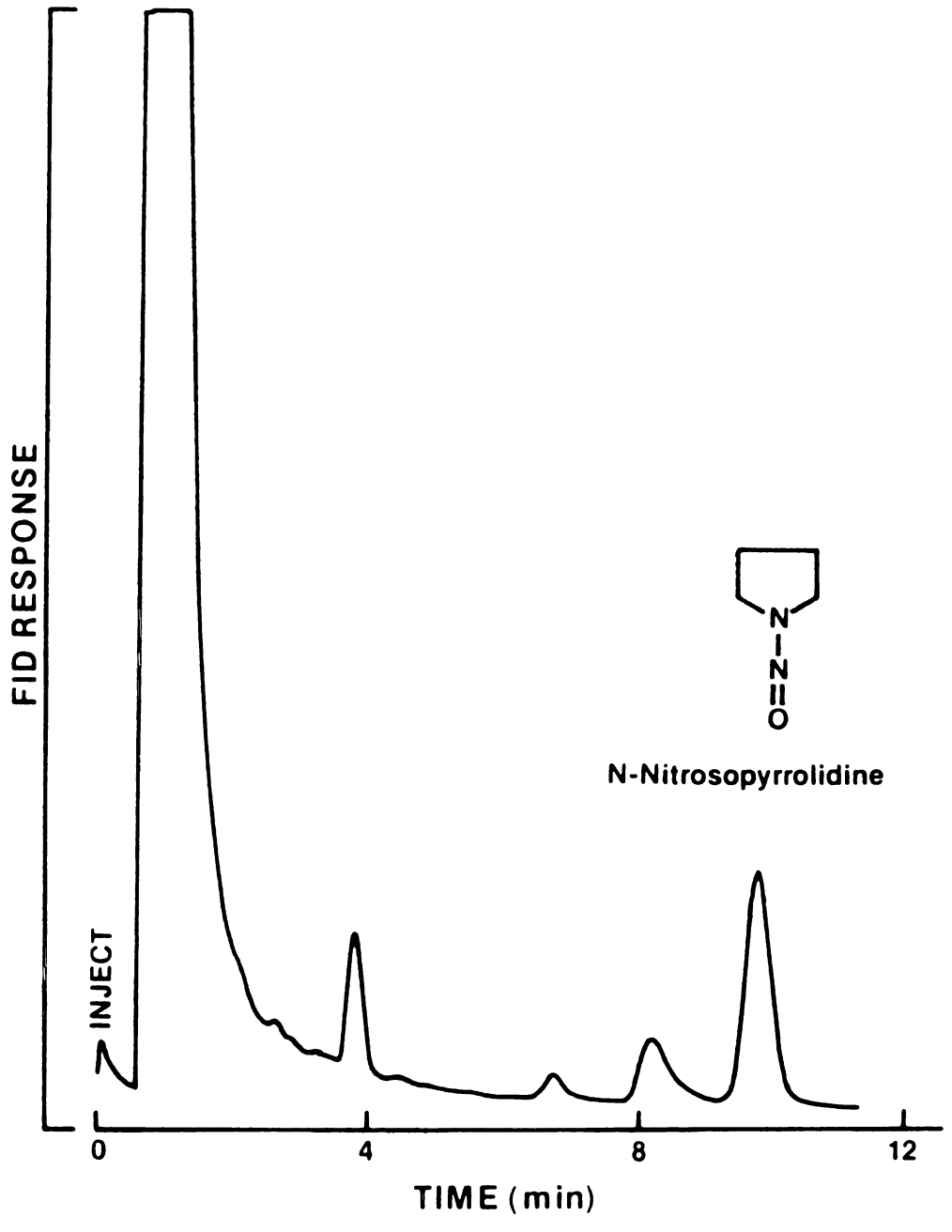
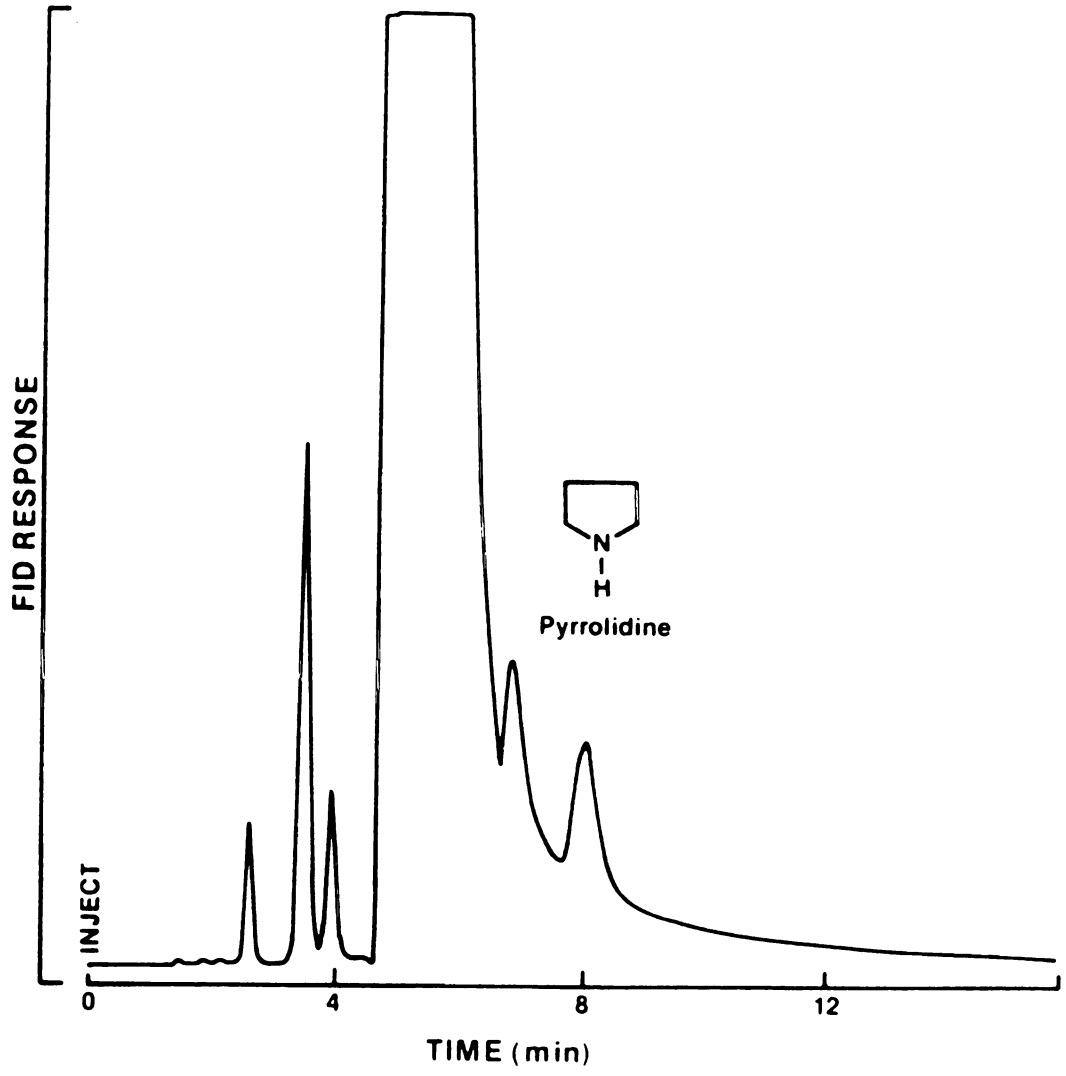

FID RESPONSE
INJECT
0
4
8
12
TIME (min)
N
N
O
N-Nitrosopyrrolidine

Figure 10. Gas chromatogram of pyrrolidine formed by heating proline (0.05 mMol) in dry model systems.


FID RESPONSE
INJECT
0
4
8
12
TIME (min)
N
H
Pyrrolidine

PYR from a heated methanolic solution containing 1,000 mg/kg of NPRO, or from a water solution containing 100 or 1,000 mg/kg of NPRO. The heat treatment was 10 min at 170°C. The major reaction product appeared to be PRO, followed by PYR and NPYR. With 100 mg/kg of NPRO, neither PRO nor PYR could be detected; however, the formation of NPYR was undeterred. Other reaction products such as pyridazine, methyl pyridazine, pyridine, pyrrole and acetic acid were identified in methanolic solutions containing 1,000 or 10,000 mg/kg of NPRO heated at 170°C for 1 h. Pyrroline was the additional product formed when 100 mg of PRO was heated in 20 mL of tetralin for 1 h at 170°C (Coleman, 1978), with PYR being the predominant product. However, when PRO was heated in water at 170°C for 1 h, no PYR was detected.

The thermal stability of NPRO and PRO has also been examined by Bharucha et al. (1979) using differential scanning calorimetry. While no thermal changes were observed with PRO in the 80-175°C range, NPRO underwent an endothermic change at 104°C indicative of its melting. This was followed by rapid decomposition with evolution of gas at 113°C. These results compared favorably with those obtained in the present study. The conversion of NPRO to NPYR is thus, relatively speaking, more facile than the transformation of PRO to PYR.

Wet model systems

NPYR formation in adipose vs lean systems. The formation of NPYR in adipose and lean systems containing PRO (0.05 mMol) and $NaNO_2$ (0.05 mMol) was investigated as a function of time and temperature (Figure 11 and Table 13). No NPYR was detected at temperatures below 120°C. Nevertheless, this temperature should not be taken as absolute since small amounts of NPYR not detectable by the FID could possibly be formed at 100°C. As indicated previously in the dry model systems, formation of NPYR increased with time and temperature at or above 120°C.

A notable difference in the amount of NPYR formed between the lean and adipose systems was observed. As much as 3 to 5 times more NPYR was formed in the adipose tissue than in lean systems at 160°C, except for the 2 min period. Overall, the ratios of NPYR formed in adipose to that produced in the lean were 1.51 ± 0.30, 1.95 ± 0.51, and 3.86 ± 1.44 at the temperatures, 120, 140 and 160°C, respectively.

Several researchers have also noted the difference in the amounts of NPYR isolated from adipose and lean components of bacon samples (Fiddler et al., 1974; Pensabene et al., 1974; Coleman, 1978). Mottram et al. (1977) reported that the ratio of the mean yields of NPYR produced in adipose, lean, and whole bacon was 12.3:1:5.2. A number of explanations for this apparent fat-preferred NPYR formation have been suggested. The greater water content of the lean may

Figure 11. Gas chromatograms of N-nitrosopyrrolidine formed by heating proline (0.05 mMol) and sodium nitrite (0.05 mMol) in (a) adipose and (b) lean systems.

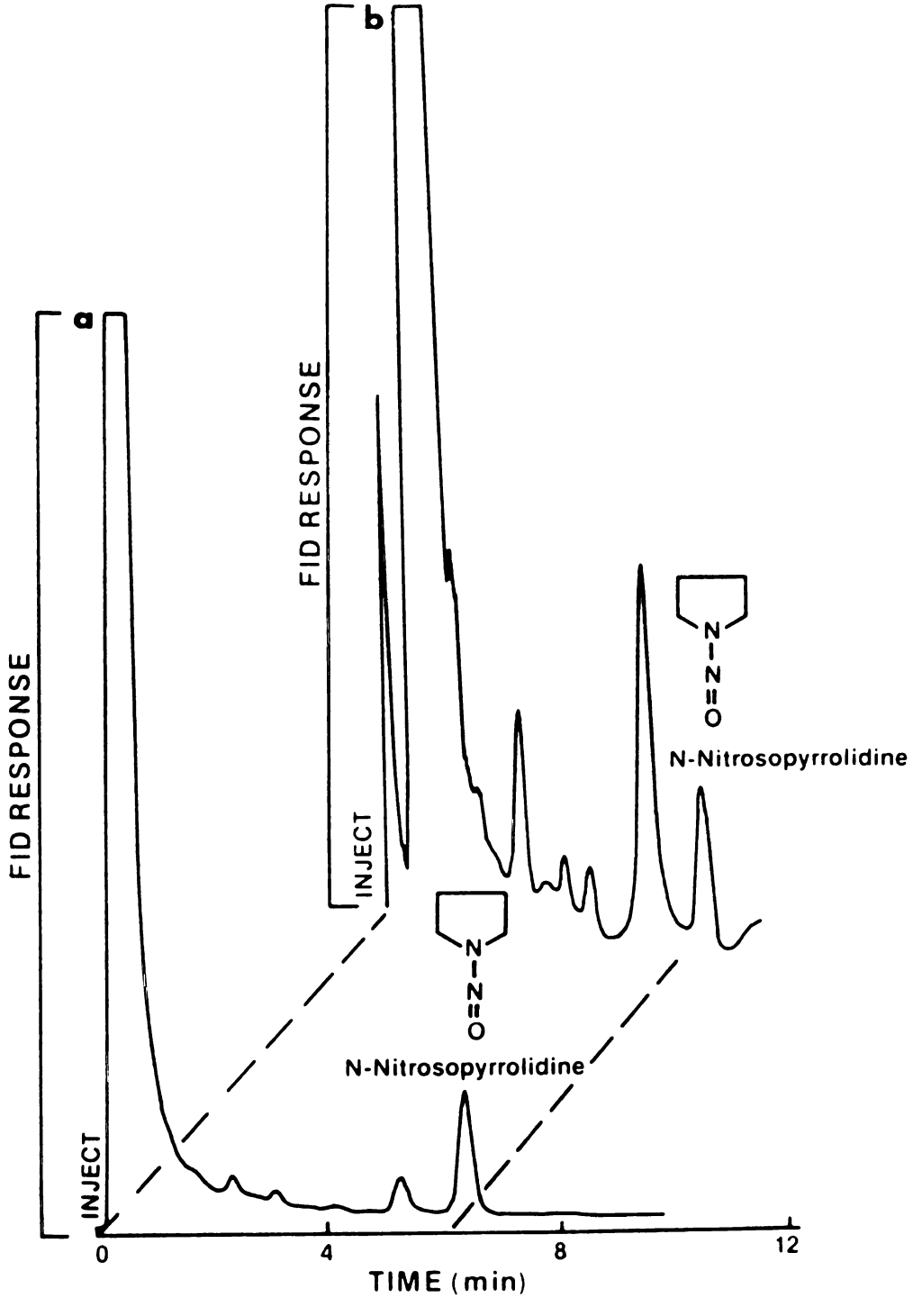

a
b
FID RESPONSE
FID RESPONSE
INJECT
INJECT
N-Nitrosopyrrolidine
N-Nitrosopyrrolidine
0
4
8
12
TIME (min)

Table 13. Effects of temperature and time on the formation of N-nitrosopyrrolidine in adipose and lean systems containing proline (0.05 mMol) and sodium nitrite (0.05 mMol).

Heating temperature (0^oC)	Heating time (min)					
	2	4	6	8	10	12
80	N.D.[a,b]	N.D.	N.D.	N.D.	N.D.	N.D.
	N.D.[c]	N.D.	N.D.	N.D.	N.D.	N.D.
100	N.D.[b]	N.D.	N.D.	N.D.	N.D.	N.D.
	N.D.[c]	N.D.	N.D.	N.D.	N.D.	N.D.
120	tr[b,d]	0.01[e,f]	0.02	0.03	0.04	0.05
	tr[c]	tr	0.01	0.02	0.03	0.04
	(--)	(--)	(2.00)[g]	(1.50)	(1.30)	(1.25)
140	tr[b]	0.05	0.11	0.15	0.22	0.26
	tr[c]	0.04	0.07	0.08	0.09	0.10
	(--)	(1.25)	(1.57)	(1.88)	(2.44)	(2.60)
160	0.05[b]	0.21	0.32	0.51	0.55	0.68
	0.04	0.07	0.09	0.10	0.11	0.13
	(1.25)	(3.00)	(3.56)	(5.10)	(5.00)	(5.23)

[a]None detectable; detection limit: 20 ng.

[b]Adipose.

[c]Lean.

[d]Trace.

[e]% yield corrected for recovery and blank.

[f]% standard deviations: ± 0.00 to 0.20 (n=2).

[g]Ratio of NPYR in adipose to NPYR in lean.

result in a higher loss of NPYR in the cooking vapors by steam distillation (Mottram et al., 1977); secondly, it may prevent the development of sufficiently high temperatures (Mottram et al., 1977), and thirdly, it may decrease the mass effect of reactant concentrations (Gray and Collins, 1977b). Alternately, the different chemical composition of adipose and lean may play a significant role (Mottram et al., 1977).

The present results did not support the hypothesis of NPYR being preferentially lost from the lean by steam distillation. Since the experiments were carried out in closed systems, the total amount of NPYR formed was quantitated. Mottram et al. (1977) have also arrived at the same conclusion. These workers have shown that the distribution of NPYR between vapor and the cooked product were similar for adipose and lean, with 12 times as much NPYR obtained from the adipose as the lean.

A more probable explanation lies in the concentration of the reactants in lean and adipose tissues. The average water contents of lean and adipose tissues were found to be in the region of 60 and 20% respectively. Therefore, the concentration of PRO and nitrite in the water phase of the adipose tissue was approximately three times than in the lean. Hence, a higher percent yield of NPYR formed in adipose at any specific temperature-time interval could be partially explained by the Law of Mass Action. In addition, Gray and Collins (1977b) have demonstrated that increased

levels of added water markedly reduced the temperature reached in the system. This effect was observed during frying of adipose and lean components in a preheated (hot) skillet.

Alternately, the difference in NPYR formation may be attributed to the differences in the chemical composition of the lean and adipose. Adipose tissue comprises 5-30% water, 2-3% protein and 60-85% lipid; the lipid is 90-99% triglyceride (Cassens et al., 1979b). In contrast, the composition of lean tissue is approximately 70% water, 20% protein, 9% fat and 1% analyzable ash (Cassens et al., 1979a). The reaction of nitrite with heme protein and the sulfhydryl groups of non-heme proteins have been well documented (Cassens et al., 1979a). Hence, the fat-preferred NPYR formation in adipose system could be explained, in part, by the greater amount of available nitrite in adipose than in the lean model systems. However, this is not true in the case of bacon system. Woolford and Cassens (1977) have shown that 73-87% of the added nitrite was in the lean portion of the bacon, while only 20-25% was in the adipose portion. In addition, Gray and Collins (1978) have reported that the average residual nitrite levels for the lean and adipose tissues were 43 and 26 mg/kg, respectively. Mottram et al. (1977), Coleman (1978) and Bharucha et al. (1979) have concluded that the non-polar liquid from the adipose tissue creates an environment conducive to NPYR formation in bacon. These authors have suggested that the formation of NPYR 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. One reaction which is known to proceed much more rapidly in non-polar solutions than under aqueous conditions is the N-nitrosation reaction by nitrogen oxides (Challis et al., 1976). Okun and Archer (1976) have provided experimental data and discussion showing that N-nitrosation of amines may be increased in the presence of micelles. More recently, the importance of morphology to the chemical reaction was discussed by Cassens et al. (1979b); they hypothesized that in bacon, the interface between the lipid globule and the cytoplasmic and connective tissue proteins may be a unique site for reaction or nitrite.

Having established that NPYR was preferentially formed in adipose rather than lean tissue as indicated by the present results and those of other researchers, further model system studies devised for the elucidation of the mode of NPYR formation in bacon, were conducted solely with adipose tissue.

N-Nitrosation of PRO and PYR in adipose systems. The formation of NPRO or NPYR from PRO (0.05 mMol) or PYR (0.05 mMol) with $NaNO_2$ (0.05 mMol) was investigated in adipose systems (Figures 12 and 13). Several interesting features were observed by following the products of these N-nitrosation reactions during the course of heat treatment. The

Figure 12. Gas chromatogram of the methyl ester of N-nitrosoproline formed by heating proline (0.05 mMol) and sodium nitrite (0.05 mMol) in adipose systems.

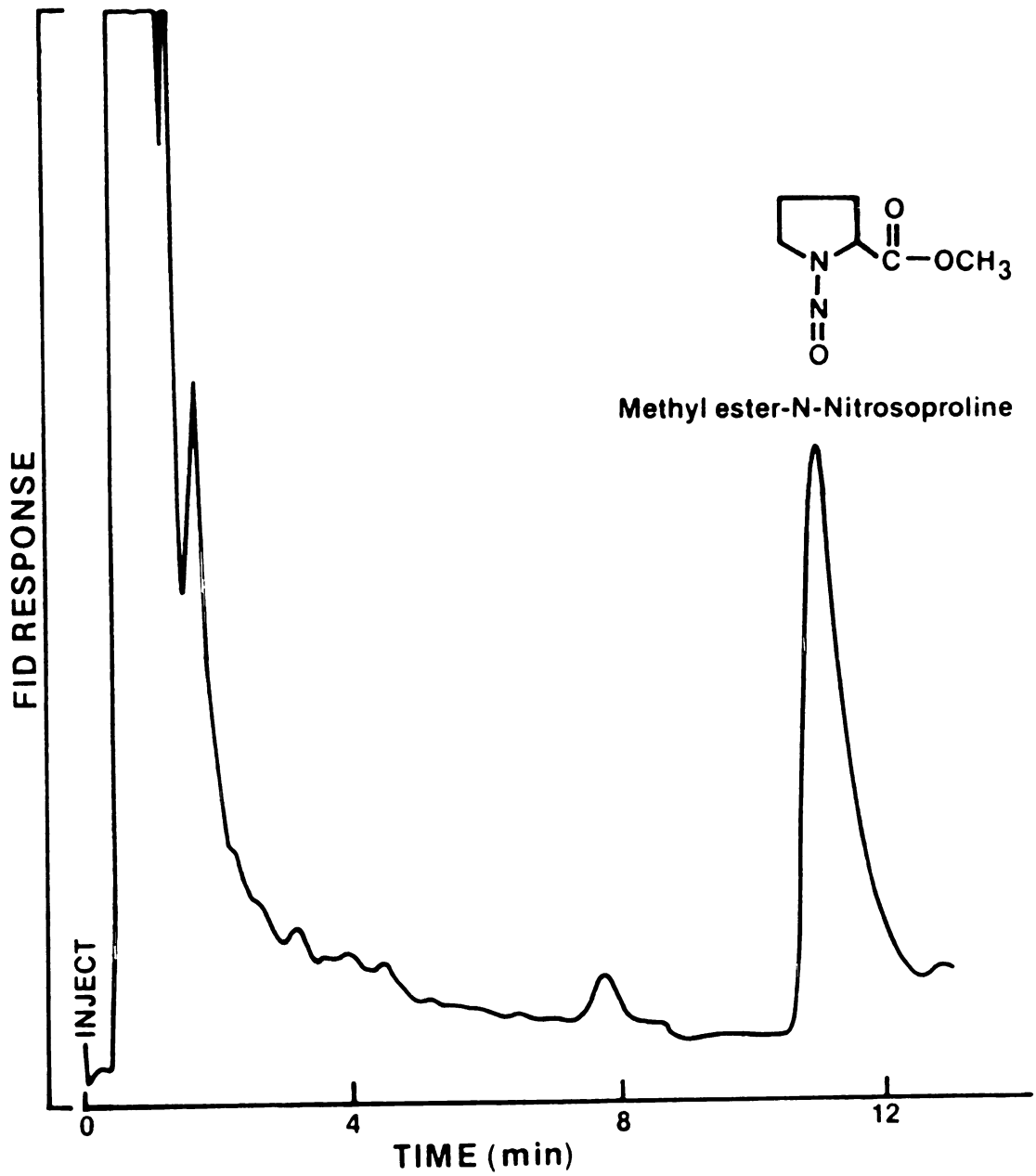
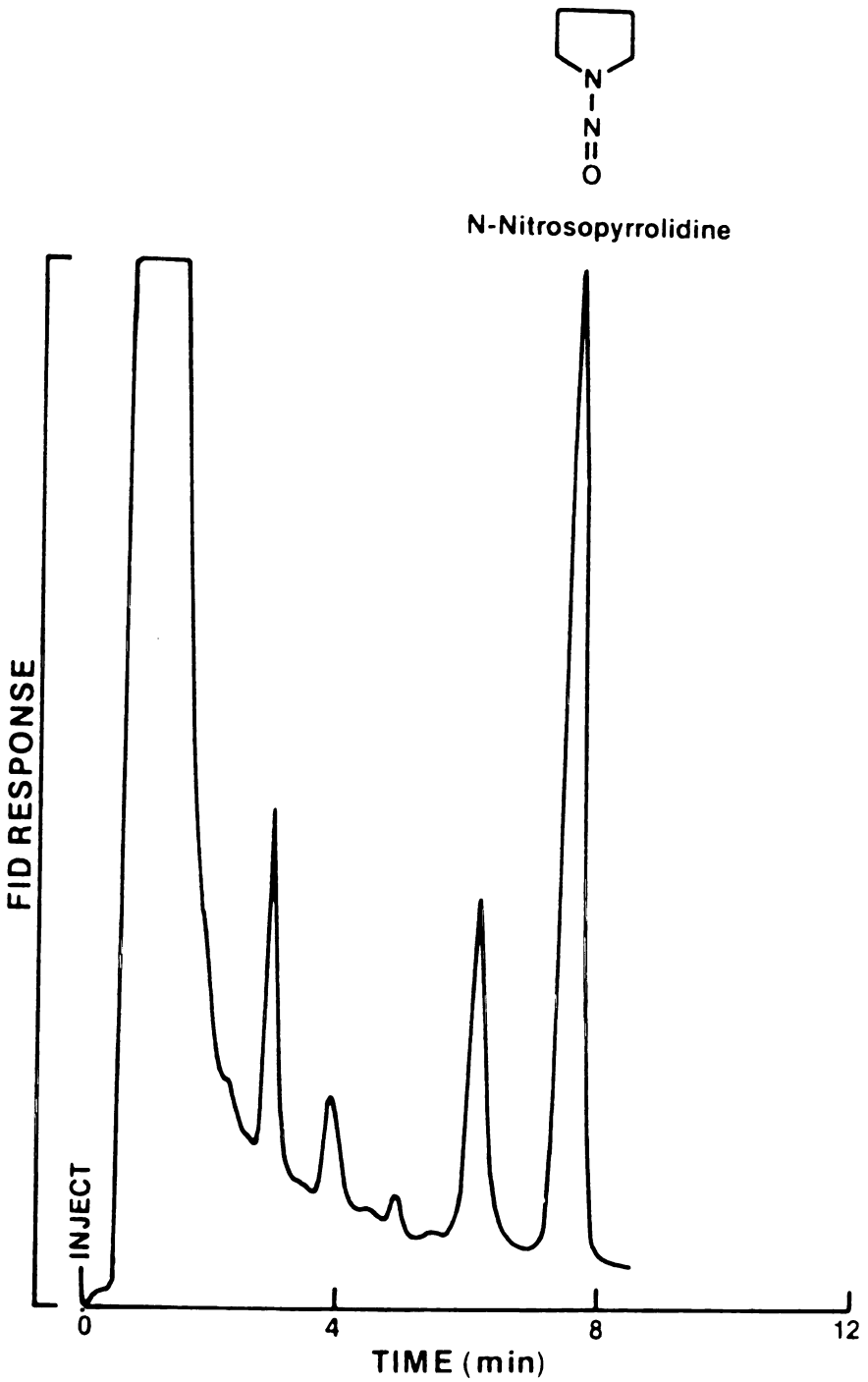

FID RESPONSE
INJECT
0
4
8
12
TIME (min)
O
C—OCH3
N
N
O
Methyl ester-N-Nitrosoproline

Figure 13. Gas chromatogram of N-nitrosopyrrolidine formed by heating pyrrolidine (0.05 mMol) and sodium nitrite (0.05 mMol) in adipose systems.


N-Nitrosopyrrolidine
FID RESPONSE
INJECT
0
4
8
12
TIME (min)

formation of NPRO was first observed to increase with heating time at 80°C and 100°C (Table 14). However, at temperatures greater than 120°C, the amount of NPRO formed reached a maximum after heating for approximately 6 min, and decreased noticeably thereafter. Based on information obtained from the dry model system studies, one can speculate that (a) at 80 and 100°C, the amount of NPRO quantitated at each time interval represented the actual amount of NPRO formed, assuming the decomposition of NPRO at 100°C was negligible, and (b) the values of NPRO obtained at 120°C or above represented the difference between NPRO formed and NPRO decomposed.

Nakamura et al. (1976) also reported that NPRO is formed from PRO and nitrite during frying of bacon. Maximum NPRO formation occurred at 125°C, above which temperature there was a gradual decrease in NPRO content up to 175°C. At 200°C, there was a rapid decrease in NPRO formation. This series of reactions involving NPRO as an intermediate product should therefore, be given due consideration in discussing the mechanism of NPYR formation in bacon.

The N-nitrosation of PYR presented a less complicated situation than its PRO counterpart. The formation of NPYR increased with heating time and temperature during the entire course of the heat treatment (Table 14). The reaction was greatly accelerated at temperatures above 140°C. Relatively speaking, the N-nitrosation of PRO was much faster than that

Table 14. Effects of temperature and time on N-nitrosation reactions in adipose systems; formation of N-nitrosoproline and N-nitrosopyrrolidine from proline (0.05 mMol) and pyrrolidine (0.05 mMol) with sodium nitrite (0.05 mMol).

Heating temperature (^{o}C)	Heating time (min)					
	2	4	6	8	10	12
80	1.8[a,c]	2.6	4.3	5.1	7.4	10.0
	0.01[b,d]	0.01	0.01	0.02	0.05	0.06
100	3.5	12.3	13.0	15.0	20.6	22.0
	0.03	0.10	0.23	0.31	0.32	0.45
120	11.2	14.0	30.0	28.1	25.5	24.3
	0.12	0.26	0.34	0.53	0.90	0.95
140	14.9	25.4	37.4	29.4	28.4	26.0
	0.38	0.98	1.70	2.40	7.41	14.3
160	20.6	35.2	44.9	41.3	39.0	10.3
	1.40	2.60	7.00	13.3	20.6	26.6

[a] % yield of NPRO corrected for recovery and blank.

[b] % yield of NPYR corrected for recovery and blank.

[c] % standard deviations: ± 0.20 to 2.00 (n=2).

[d] % standard deviations: ± 0.01 to 0.90 (n=2).

of PYR, a conclusion also reached by Mirvish (1975).

Decarboxylation of PRO and NPRO in adipose systems. Results of the formation of PYR and NPYR from PRO (0.05 mMol) and NPRO (0.05 mMol), respectively, in heated adipose systems are given in Table 15. Typical gas chromatograms of the decarboxylation products in adipose systems are presented in Figures 14 and 15.

The decarboxylation reactions were initiated at temperatures above 100°C, the reaction products increased with increasing temperature and time. Unlike the dry model system study, PRO decarboxylated at a much lower temperature. This observation has previously been reported by Coleman (1978). A contributing factor could be the excellent heat transfer medium provided by the non-polar lipids.

The results in Table 15 indicate that the decarboxylation of PRO to PYR was more pronounced at the higher temperatures, i.e. temperature at or above 160°C. Nakamura et al. (1976) have shown that decarboxylation of PRO to produce PYR in bacon generally occurred in the range of 175-225°C.

On the other hand, NPRO was more readily decarboxylated to NPYR upon heating as reported by Bharucha et al. (1979) and, again in the dry model system studies. However, the decarboxylation reaction of NPRO to NPYR was less specific than that of PRO to PYR. At least three other products were isolated when NPRO was subjected to thermal stress. This explained the ratios of % NPYR to % PYR being less than 1

Table 15. Effects of temperature and time on decarboxylation reactions in adipose systems; formation of N-nitrosopyrrolidine from N-nitrosoproline (0.05 mMol) and pyrrolidine from proline (0.05 mMol).

Heating temperature (^{o}C)	Heating time (min)					
	2	4	6	8	10	12
80	N.D.[a]	N.D.	N.D.	N.D.	N.D.	N.D.
	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
100	tr[b]	tr	tr	tr	tr	tr
	N.D.	N.D.	N.D.	N.D.	0.05[c,e]	0.06
120	0.02[d,e]	0.05	0.06	0.07	0.15	0.19
	tr	0.06	0.08	0.11	0.17	0.22
	(--)	(0.83)[f]	(0.75)	(0.64)	(0.88)	(0.86)
140	0.07	0.12	0.14	0.16	0.23	0.32
	0.09	0.13	0.19	0.24	0.33	0.44
	(0.77)	(0.92)	(0.74)	(0.66)	(0.69)	0.73)
160	0.19	0.33	0.53	0.65	0.74	0.78
	0.48	0.53	0.94	1.10	1.30	2.30
	(0.40)	(0.62)	(0.56)	(0.59)	(0.56)	(0.34)

[a]None detectable; detection limit 20 ng.

[b]Trace.

[c]% yield of PYR corrected for recovery and blank.

[d]% yield of NPYR corrected for recovery and blank.

[e]% standard deviations: ± 0.00 to 0.20 (n=2).

[f]Ratio of % NPYR to % PYR.

Figure 14. Gas chromatogram of N-nitrosopyrrolidine formed by heating N-nitrosoproline (0.05 mMol) in adipose systems

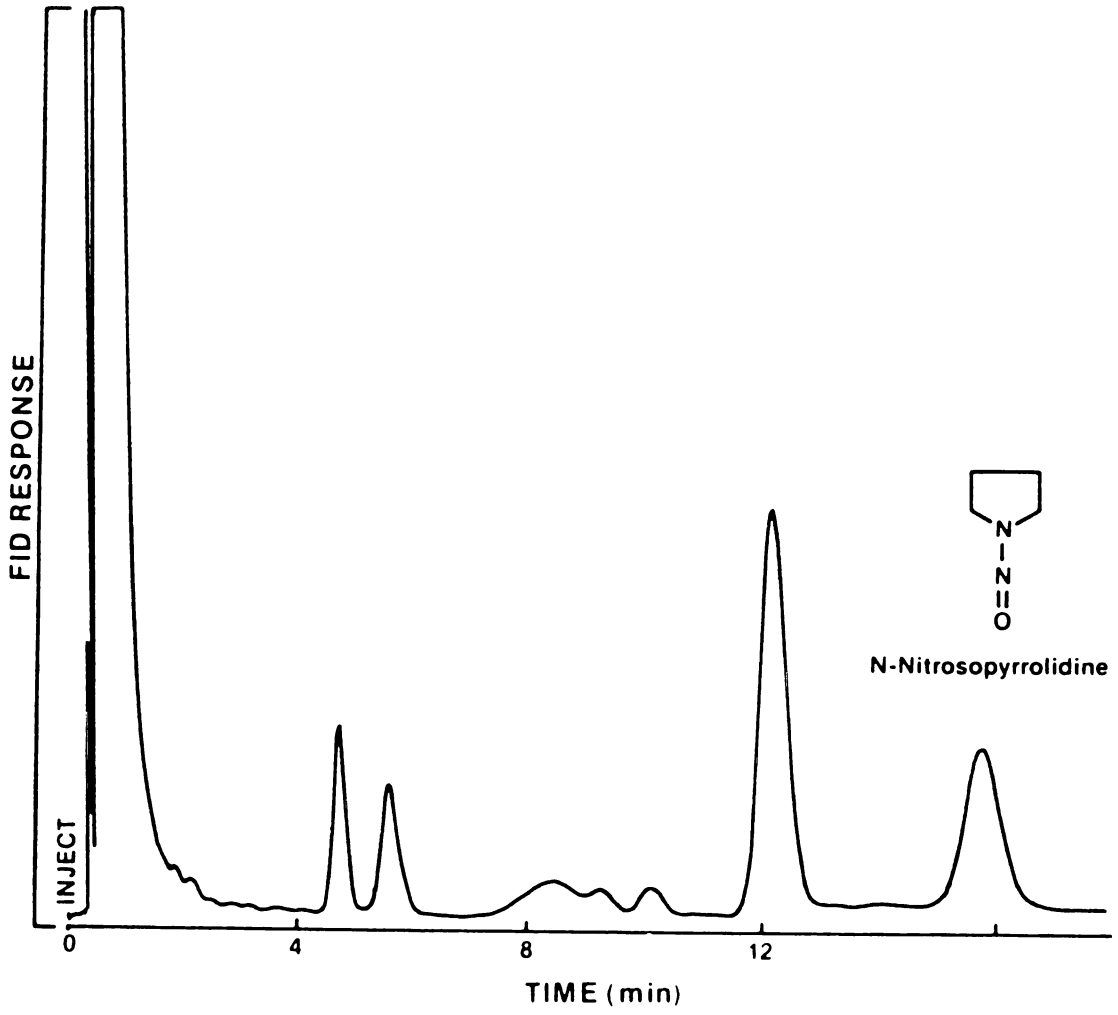
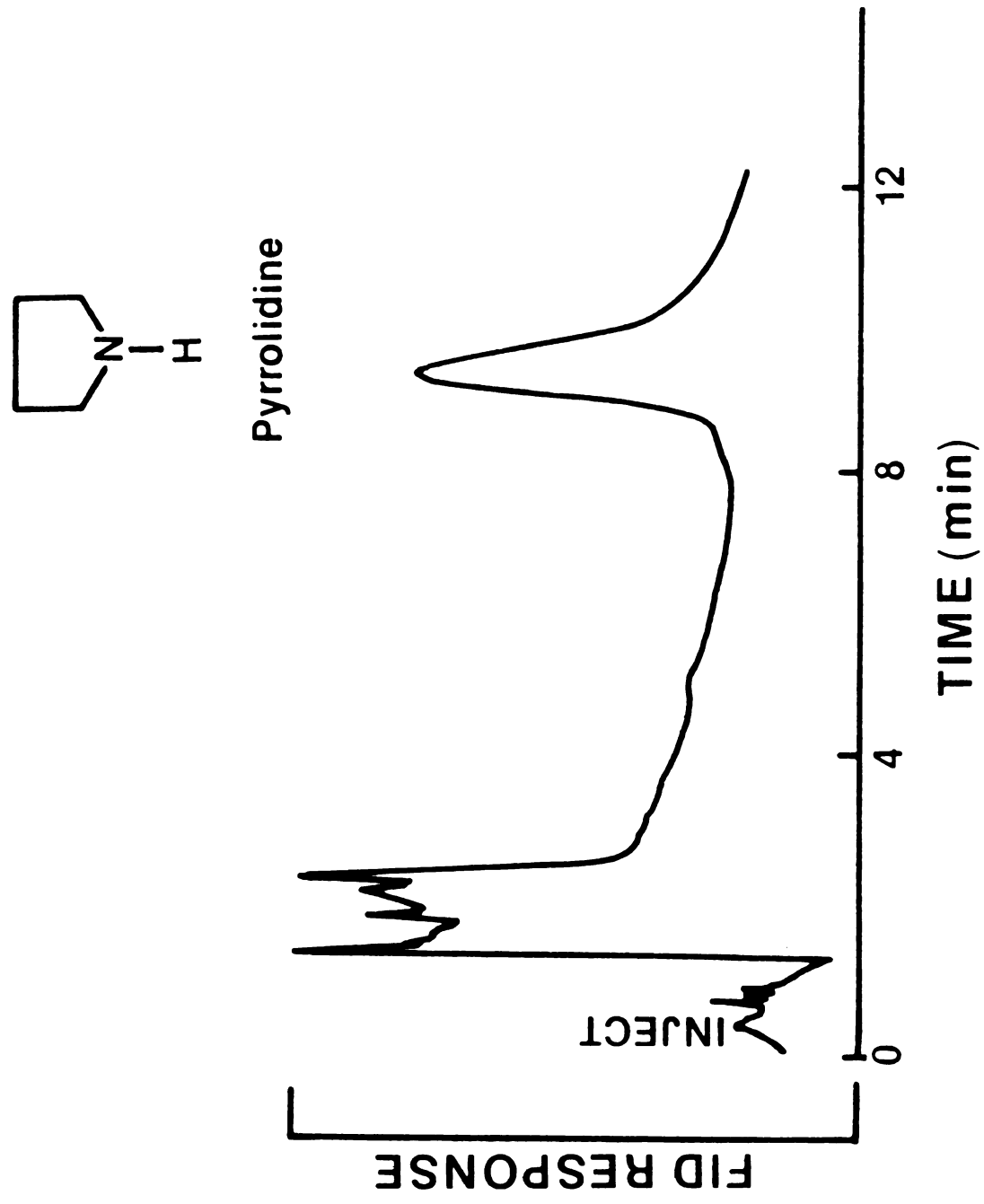

FID RESPONSE
INJECT
0
4
8
12
TIME (min)
N
N
O
N-Nitrosopyrrolidine

Figure 15. Gas chromatogram of pyrrolidine formed by heating proline (0.05 mMol) in adipose systems.


Pyrrolidine
N
H
FID RESPONSE
INJECT
0
4
8
12
TIME (min)

(Table 15). Overall, the ratios of % NPYR to % PYR were 0.79 ± 0.09, 0.75 ± 0.09, and 0.51 ± 0.10 at the temperatures, 120, 140 and 160°C, respectively.

Kinetic Data of N-Nitrosation and Decarboxylation Reactions in Adipose Systems

The first and third order rate constants, as a function of time and temperature, for the decarboxylation and N-nitrosation reactions were investigated. A least square criterion, KINFIT 4, proposed by Dye and Nicely (1971) was used to estimate the rate constants. The activation energy was determined graphically (Figure 16). The kinetic studies were followed by the appearance of reaction products.

Results of these studies (Table 16, 17) strongly indicate that in adipose systems, PRO was more likely to N-nitrosate than decarboxylate in the range of temperatures (80-165°C) encountered during the frying of bacon. In general the pathway involving N-nitrosation of PRO, followed by decarboxylation of NPRO to NPYR, requires an overall activation energy of 30 Kcal/mole. However, an estimated value of 40 Kcal/mole is required for pathway involving PYR as the intermediate compound.

Fan and Tannenbaum (1972) determined the first order rate constants for the decomposition of N-nitrosamino acids at 110°C and various pH values. The rate constant at pH 5.5 for NPRO was found to be 8.02×10^{-5} min^{-1} with a half-life

Figure 16. Arrhenius plot of ln k versus $\frac{1}{T\ ^{o}K}$.

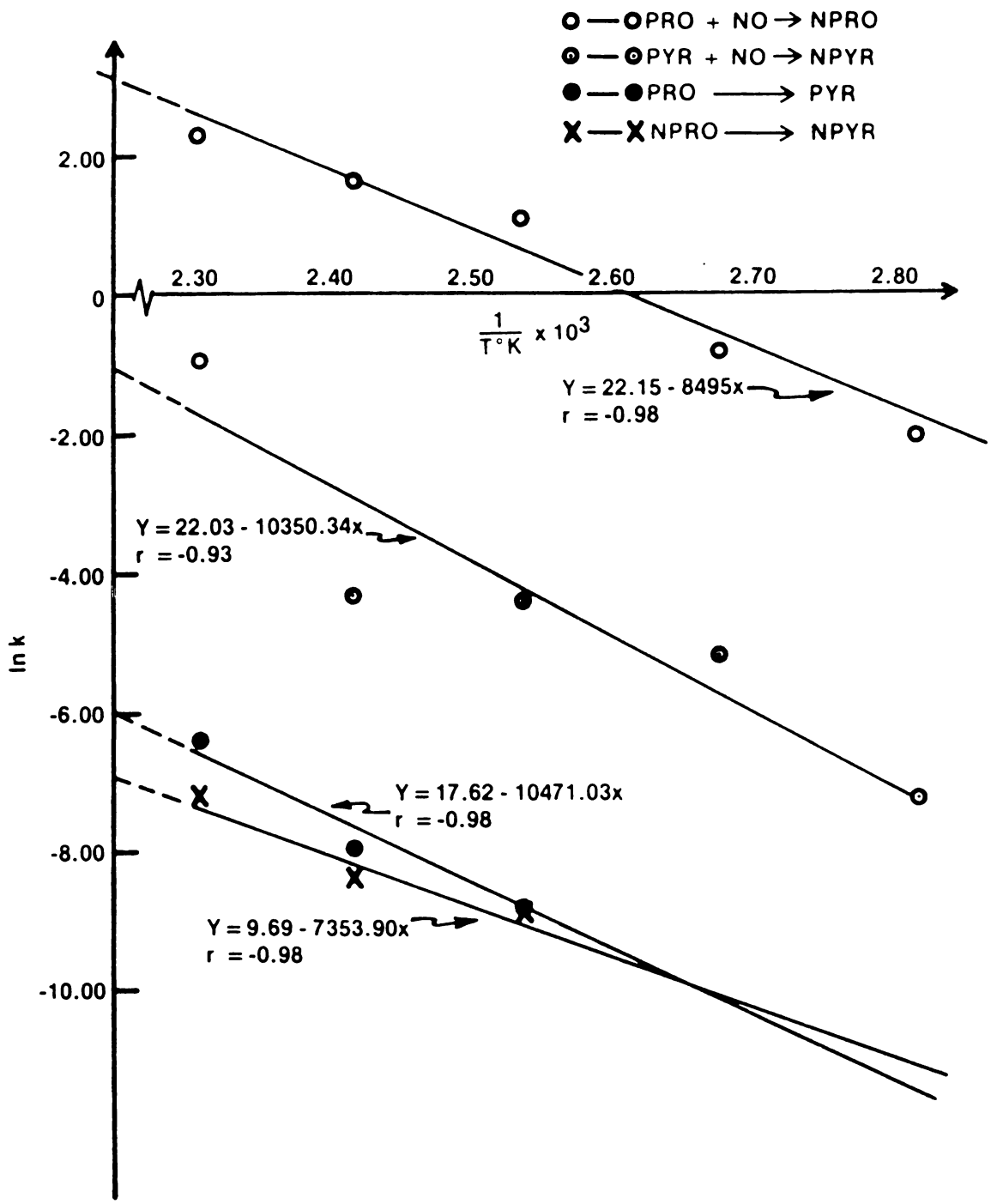

O—O PRO + NO → NPRO
O—O PYR + NO → NPYR
●—● PRO → PYR
X—X NPRO → NPYR
2.00
0
-2.00
-4.00
-6.00
-8.00
-10.00
2.30
2.40
2.50
2.60
2.70
2.80
1/T°K x 10^3
ln k
Y = 22.15 - 8495x
r = -0.98
Y = 22.03 - 10350.34x
r = -0.93
Y = 17.62 - 10471.03x
r = -0.98
Y = 9.69 - 7353.90x
r = -0.98

Table 16. Kinetics data for the N-nitrosation of proline and pyrrolidine in adipose systems at various temperatures.

Temperature (°C)	PRO + NO ⟶ NPRO			PYR + NO ⟶ NPYR		
		k			k	
	$t_{1/2}$ min	M^{-2} min^{-1}	percent^{-2} min^{-1}	$t_{1/2}$ min	M^{-2} min^{-1}	percent^{-2} min^{-1}
80	178	1.35×10^{-1}	8.43×10^{-7}	3.69×10^{4}	6.59×10^{-4}	4.12×10^{-9}
110	54.5	4.40×10^{-1}	2.73×10^{-6}	2.80×10^{3}	5.71×10^{-3}	3.57×10^{-8}
120	7.95	3.02	1.89×10^{-5}	1.94×10^{3}	1.24×10^{-2}	7.76×10^{-8}
140	4.56	5.26	3.29×10^{-5}	1.79×10^{3}	1.34×10^{-2}	8.35×10^{-7}
160	2.57	9.33	5.83×10^{-5}	62	3.86×10^{-1}	2.41×10^{-6}
E_a (Kcal/mole)		16.87			20.56	

Table 17. Kinetics data for the decarboxylation of proline and N-nitrosoproline in adipose systems at various temperatures.

Temperature (°C)	NPRO ⟶ NPYR		PRO ⟶ PYR	
	$t_{1/2}$ min	k min^{-1}	$t_{1/2}$ min	k min^{-1}
80[a]	51.3×10^{3}	1.35×10^{-5}	12.9×10^{4}	5.35×10^{-6}
100[a]	16.7×10^{3}	4.14×10^{-5}	26.3×10^{3}	2.64×10^{-5}
120	5.13×10^{3}	1.35×10^{-4}	4.85×10^{3}	1.43×10^{-4}
140	2.84×10^{3}	2.44×10^{-4}	2.04×10^{3}	3.40×10^{-4}
160	9.36×10^{3}	7.40×10^{-4}	4.28×10^{2}	1.62×10^{-3}
E_a (Kcal/mole)	14.61		20.80	

[a]Calculated values.

value of 8.64×10^3 min. Using an activation energy value of 14.61 Kcal/mole (Table 17), the rate constant for NPRO decarboxylation in adipose systems (pH 5.6-5.8) was estimated to be 7.0×10^{-5} min^{-1} with a half-life value of 9.9×10^3 min.

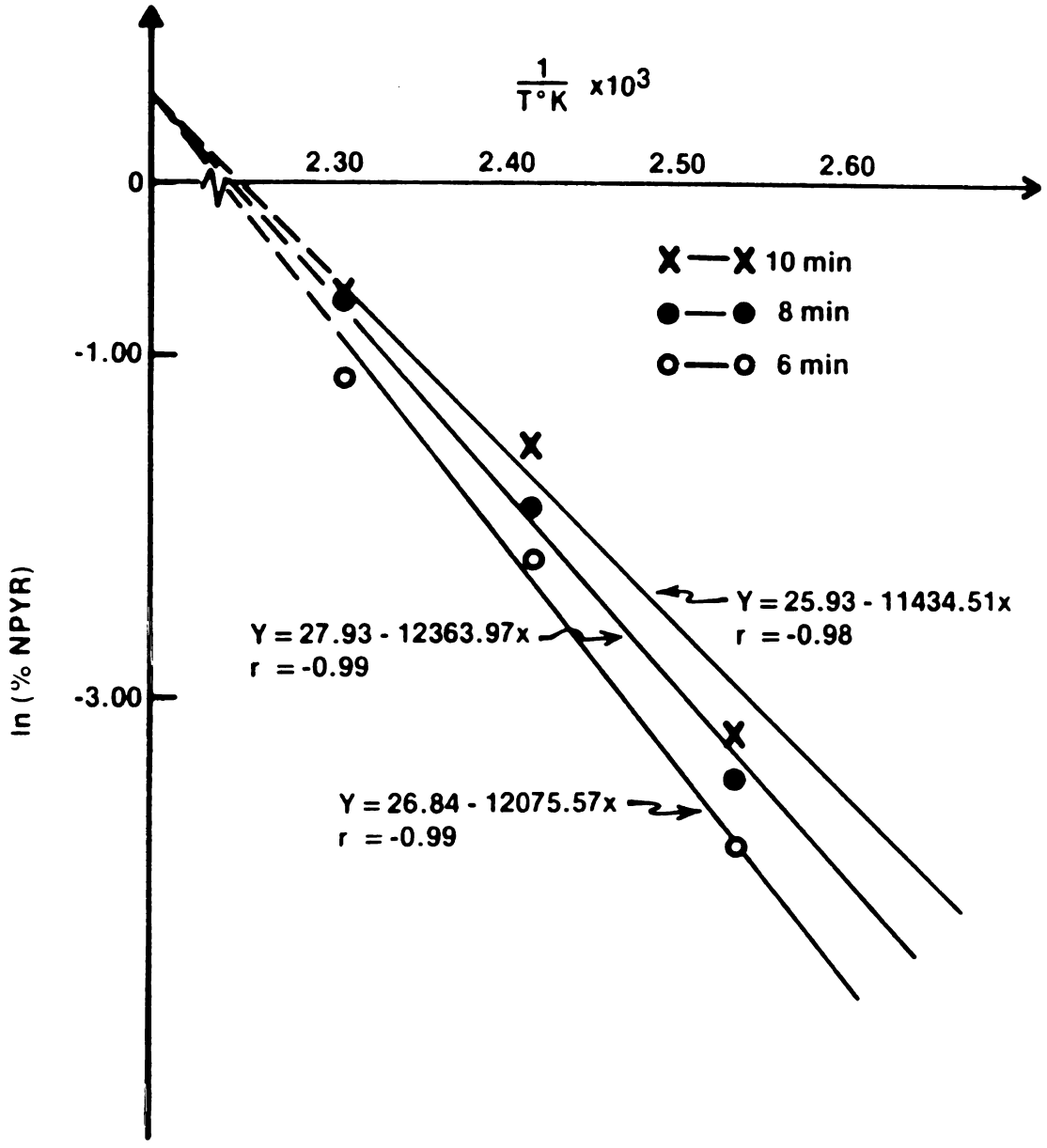
An easier comparison of the rates of reactions could be made by comparing the half-life values. In view of the results obtained, N-nitrosation of PRO occurred most readily, followed by N-nitrosation of PYR, then the decarboxylation reactions. Mirvish et al. (1973) reported a rate constant of 2.2 M^{-2} min^{-1} for the N-nitrosation of PRO at 25°C at pH 2.5. A rate constant of 0.318 M^{-2} min^{-1} for the N-nitrosation of PYR at pH 3.0 (25°C) was later reported (Mirvish, 1975).

The third order rate constants for the N-nitrosation reactions was calculated using the stoichiometric reactant concentration. The resulting k values, therefore, represent the pH-dependent third order rate constants.

The overall activation energy for the formation of NPYR from PRO and nitrite as estimated from a plot of ln (% NPYR) against the reciprocal of absolute temperature (Coleman, 1978) is illustrated in Figure 17. This value was calculated as 23.68 Kcal/mole which compared favorably with the reported value, 27.2 Kcal/mole of Coleman (1978).

For better approximation of the kinetic data of the N-nitrosation of PRO, the experiment should be conducted at

Figure 17. Arrhenius plot of ln (% NPYR) versus $\frac{1}{T\ ^{o}K}$ for the formation of N-nitrosopyrrolidine from proline (0.05 mMol) and sodium nitrite (0.05 mMol) in heated adipose systems.


1/T°K x10³
2.30
2.40
2.50
2.60
0
-1.00
-3.00
ln (% NPYR)
10 min
8 min
6 min
Y = 25.93 - 11434.51x
r = -0.98
Y = 27.93 - 12363.97x
r = -0.99
Y = 26.84 - 12075.57x
r = -0.99

temperatures below 100°C, preferably at a temperature lower than 80°C to avoid complication due to the decomposition of NPRO.

General Discussion

In the preliminary experiments with bacon frying, the effects of frying procedures (preheated vs cold skillet) and compositional factors (adipose vs lean) on the final temperatures attained in fried bacon were demonstrated. In confirmation of the suggestion of Scanlan (1975), and as demonstrated by Mottram et al. (1977) and Coleman (1978), higher temperatures were reached in the adipose tissue than in the lean during frying. In addition, model system studies have indicated that 3 to 5 times as much NPYR was isolated from adipose systems containing PRO and $NaNO_2$ than from lean systems containing the same precursor. This observation is in agreement with those of Fiddler (1974), Mottram et al. (1977) and Coleman (1978).

It has also been well established that pan frying of bacon results in more NPYR formation than either microwave cooking (Herring, 1973; Pensabene et al., 1974) or grilling (Bharucha et al., 1979). The latter authors have demonstrated that both frying temperature and time clearly influence the levels of NPYR in cooked bacon. Pensabene et al. (1974) showed that bacon samples prepared from one belly formed no NPYR when fried for 105 min at 99°C, while

samples from the same belly, fried to the same "doneness" at 204°C for 4 min, produced 17 μg/kg of NPYR. Therefore, it is tempting to speculate that bacon samples fried in an initially cold skillet may result in lower NPYR formation than those fried in a preheated skillet. This is an area which requires further investigation.

Table 18 summarizes the data (Tables 14-17) of experiments with adipose systems, designed to simulate the formation of NPYR in fried bacon. The relationship between heating temperature and time, and the possible pathways of NPYR formation in bacon was clearly demonstrated. About 60-97% of the NPYR isolated from heated adipose systems containing PRO and $NaNO_2$ could not be explained by the pathway involving PYR as an intermediate compound. These results implied that, if the alternate pathway involving NPRO was the major route of NPYR formation in bacon, N-nitrosation of PRO would have to proceed to near completion ($\geq$ 90%) during the prescribed thermal treatment. Indeed, greater than 90% of NPRO formation could be approximated from the experimental data (Tables 14-17), using the third order rate equation for N-nitrosation of PRO (Table 19). However, due to the complexity of reactions involved at elevated temperatures (120-160°C) and prolonged heating (8-12 min), a maximum of 90% yield of NPRO was used in the estimation of the percent NPYR formed via NPRO.

Table 18. Comparison of yields of N-nitrosopyrrolidine formation between pathways involving N-nitrosoproline and pyrrolidine.

Heating temperature (°C)	Heating time (min)	Percent N-nitrosopyrrolidine isolated (I)[a]	via NPRO (A)[b]	via PYR (B)[b]	$\%\frac{A}{I}$	$\%\frac{B}{I}$
120	2	tr[c]	--	--	--	--
	4	0.01	0.02	0.0002	100	2.0
	6	0.02	0.03	0.0004	100	2.0
	8	0.03	0.05	0.0006	100	2.0
	10	0.04	0.11	0.001	100	2.5
	12	0.05	0.15[d]	0.002	100	4.0
				Average:	100	2.5
140	2	tr	--	--	--	--
	4	0.05	0.06	0.003	100	6.0
	6	0.11	0.07	0.007	63.6	6.4
	8	0.15	0.16[d]	0.01	100	6.7
	10	0.22	0.21[d]	0.03	95.5	13.6
	12	0.26	0.28[d]	0.05	100	19.2
				Average:	91.8	10.4
160	2	0.05	0.08	0.01	100	20.0
	4	0.21	0.20	0.04	95.2	19.1
	6	0.32	0.36	0.11	100	34.4
	8	0.51	0.56[d]	0.20	100	39.2
	10	0.55	0.66[d]	0.32	100	58.2
	12	0.68	0.75[d]	0.51	100	75.0
				Average:	99.2	41.0

[a] See Table 13.

[b] Approximated using data in Tables 14-17.

[c] Trace.

[d] Estimated using 90% as a maximum yield of NPRO formation.

Table 19. Percent formation of N-nitrosoproline from proline (0.05 mMol) and sodium nitrite (0.05 mMol) in adipose systems.

Heating temperature (°C)	Heating time (min)	k percent^{-2} min^{-1}	% NPRO[a] formed
120	6	1.85×10^{-5}	38.9
	8		56.2
140	6	3.29×10^{-5}	48.4
	8		92.6
160	6	5.83×10^{-5}	58.5
	8		94.3

[a]Estimated using third order rate equation for N-nitrosation of proline.

Several interesting features were observed in the study of NPYR formation in adipose systems. As seen in Table 18, the formation of NPYR via PYR became increasingly important with increased temperature (≥160°C). Additionally, in following the loss of PRO versus that of NPRO during heating, PYR appeared to be the major decomposition product (Figure 10), whereas NPRO decomposed to several other products beside NPYR (Figure 9).

Nakamura et al. (1976) showed that decarboxylation of PRO to PYR in bacon occurred extensively in the temperature range 175-225°C. In model system studies, Coleman (1978) also reported the ease of PRO decarboxylating to form PYR when heated at 170°C for 1 h. These authors then theorized that the pathway involving PYR was the major route of NPYR formation in bacon. However, as indicated earlier (Figure 7), bacon samples rarely attained a temperature greater than 164 ± 3°C when fried at 171°C in preheated skillet. More importantly, bacon samples only arrived at temperatures in the region of 160°C during the last 1-2 min of frying.

Assuming a PRO content of 21-80 mg/kg (or 173-691 μM/kg) (Bharucha et al., 1979), a residual nitrite content of 10-50 mg/kg (or 145-725 μM/kg) and a 90% N-nitrosation of PRO (experimental data) in bacon, approximately 131-623 μM/kg of NPRO may be formed during the frying process. Bharucha et al. (1979) estimated a 0.16% yield of NPYR from the decarboxylation of NPRO under bacon frying conditions, and consequently,

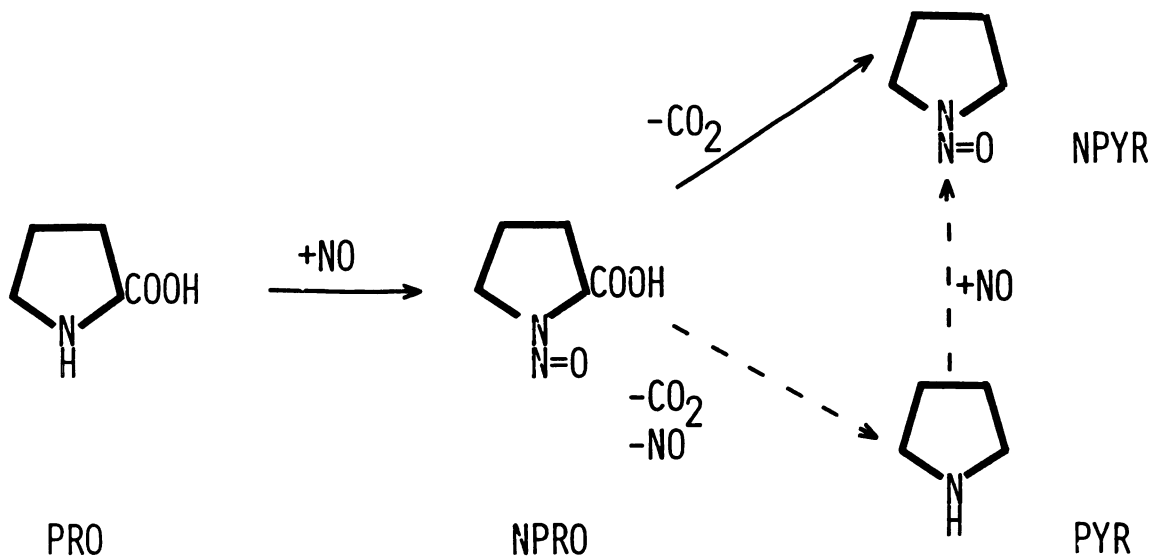
a yield of 0.21-1.0 μM/kg (or 21.2-100 μg/kg) of NPYR may be formed via the pathway involving NPRO. Hence, the existence of NPYR in bacon could be explained by the decarboxylation of NPRO. As pointed out by Bharucha et al. (1979), the amount of preformed NPRO (40 μg/kg) in raw bacon could not account for the presence of NPYR in the μg/kg range normally found in cooked bacon and therefore, cannot be considered the primary precursor of NPYR. Other investigators have come to similar conclusions (Hansen et al., 1977; Gray and Collins, 1978; Pensabene et al., 1979b). However this by no means rules out the intermediacy of NPRO which could be formed at the higher temperatures attained during the frying process (Gray and Collins, 1978; Bharucha et al., 1979).

In a recent study on the occurrence of N-nitrosamino acids in cured meat and their effect on the formation of N-nitrosamines during heating, Janzowski et al. (1978) concluded that the decarboxylation of N-nitrosamino acids is not the essential pathway of N-nitrosamines formation. However, these authors have failed to discuss the possible formation of NPRO during the cooking process.

Obviously, the extent of N-nitrosation of PRO in the presence of other curing ingredients during bacon frying is a key issue which necessitates further investigation. The above estimation of 21-100 μg/kg of NPYR formed via NPRO in fried bacon is a projection based on the results of the model system studies. This served only to illustrate the

importance of NPRO formation during the frying of bacon. Nevertheless, approximately 10-40% of NPRO formed during frying is sufficient to account for the presence of NPYR in bacon.

Based on the results obtained from the current study, a more likely pathway for the formation of NPYR in bacon is proposed as follows:



The ease of denitrosation and decarboxylation of NPRO has been demonstrated by Fan and Tannenbaum (1972) and Coleman (1978). Hence, the formation of PYR from NPRO as a function of temperature and time requires further investigation. Coleman (1978) and Bharucha et al. (1979) have postulated that during the frying of bacon, N-nitrosation of PRO

proceeded via a free radical mechanism and occurred at temperatures greater than 100°C, after the bulk of water is removed from the system. However, the results obtained from model system studies (Table 14) indicated that N-nitrosation of PRO occurred even at temperatures below 100°C.

Further work on the N-nitrosation of PRO and PYR and the decarboxylation of PRO and NPRO in bacon systems is needed before the mechanism of NPYR formation can be firmly established. Such an understanding is required for the suppression or elimination of N-nitrosamines formation in cooked bacon.

SUMMARY AND CONCLUSIONS

The effects of frying procedures (preheated vs cold skillet) and compositional factors (adipose vs lean) on the temperatures attained in fried bacon were investigated using a thermocouple. The mechanism(s) of NPYR formation in model systems simulating the pan frying conditions of bacon was explored. The reaction products of these model system studies were analyzed by GC. The effects of adipose versus lean tissues on the formation of NPYR in model systems were also investigated.

As a result of these studies, several conclusions pertaining to the formation of NPYR in bacon are reached. These are summarized below:

1. The temperatures reached in bacon during the frying process were significantly affected by (a) the methods of frying--hot versus cold skillet ($p \leq 0.01$) and (b) the composition of bacon samples--adipose versus lean components ($p \leq 0.10$).

2. With a preheated skillet set at 171^{o}C, the final temperature reached in whole bacon samples at the end of the 6 min frying time is about 164 $\pm$ 3^{o}C.

3. The formation of NPYR in heated adipose systems increases with increasing heating temperature (120-160^{o}C)

and time (2-12 min).

4. Approximately 3 to 5 times as much NPYR is found in the heated adipose system containing added PRO and $NaNO_2$ (0.05 mMol of each reactant) than in the lean counterpart.

5. The N-nitrosation of PRO or PYR in adipose model systems occurs readily in the temperature range, 80-160^{o}C.

6. The decarboxylation of PRO or NPRO in adipose model systems proceeds only at 100^{o}C or above.

7. The rates of the above reactions (5 and 6) are such that N-nitrosation of PRO > N-nitrosation of PYR > decarboxylation of PRO or NPRO.

8. The results from model system studies lend credence to the pathway involving NPRO, in the formation of NPYR in bacon. This is in agreement with the observations of Hwang and Rosen (1976) and Bharucha et al. (1979).

APPENDIX

APPENDIX

Preparation of N-nitrosoproline

500 mg L(-)PRO is added in 25 mL water. A tenfold excess of $NaNO_2$ is then added and the solution is acidified to pH 3 with hydrochloric acid. The reaction is allowed to proceed in the dark, while being stirred at room temperature for 18 h. The mixture is then acidified to pH 1 to protonate the acid and facilitate solvent extraction. The water is evaporated under reduced pressure and the residual solids extracted with two 25 mL portions of methylene chloride. The extract is dried with sodium sulfate, filtered and finally methylene chloride is removed using the rotary evaporator.

Preparation of ethereal alcoholic solutions of diazomethane

Ethanol (95%, 25 mL) is added to solution of potassium hydroxide (5 g) in water (8 mL) in a 100 mL distilling flask fitted with dropping funnel and an efficient condenser set downward for distillation. The condenser is connected to a receiving flask cooled to $0^{o}C$.

The flask containing the alkali solution is heated in a water bath to $65^{o}C$, and a solution of 21.5 g (0.1 mole) of $Diazald_R$ in about 200 mL of ether is added through the

dropping funnel in about 25 min. The rate of distillation should about equal the rate of addition. When the dropping funnel is empty, another 40 mL of ether is added slowly and the distillation is continued until the distilling ether is colorless. The combined ethereal distillate contains about 3 g of diazomethane.

Reaction rate laws

a. First order reaction.

$$kt = x$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k}$$

b. Third order reaction.

$$kt = \frac{1}{2(a - x)^2}$$

$$t_{\frac{1}{2}} = \frac{3}{2ka^2}$$

a: original concentration

x: concentration of product formed

Arrhenius equation

$$\ln k = \frac{-E_a}{RT} + \text{constant}$$

R: 1.9872 $Kcal^{-1}$ mol^{-1}

T: absolute temperature

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BIBLIOGRAPHY

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