The Influence of Microwave Heating on the Formation of N-Nitrosamines in Bacon

by

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ABSTRACT

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Bacon was prepared using conventional frying and microwave cooking in transparent and susceptor packages in a 700 W microwave oven. Bacon slices were prepared to three degrees of doneness, undercooked, overcooked and similar to standard pan frying (3 min on each side at 340°F), by controlling the time in the microwave oven. The package interfacial temperature and bacon temperature were monitored using a Luxtron fluoroptic probe system. Final bacon temperatures of 167°C (330°F) and 143°C (290°F) were achieved for bacon samples cooked to the same degree of doneness as fried bacon in the susceptor (2.5 min) and transparent (3 min) packages, respectively. Compared to frying, less N-nitrosopyrrolidine (NPYR) was present in bacon prepared either in the transparent or susceptor packages. Greater concentrations of NPYR were found in bacon prepared on the susceptor trays compared to bacon prepared in the transparent packages. Increased NPYR formation in pan-fried bacon was observed with increased nitrite concentrations in the raw product. However, no significant increase in NPYR concentrations was found in microwaved bacon prepared with the higher level of ingoing nitrite.

Dedicated to My Family.

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INTRODUCTION

Convenience in food preparation makes the microwave oven a necessary component for most households. A significant time-savings can be achieved with microwave "in-package" cooking. With penetration of microwave ovens in the United States approximately 80%, and retail sales of microwave foods several billion dollars, the development of new microwave foods has become an increasingly important Research and Development focus for many food companies (Anonymous, 1988).

Insufficient crisping and browning have been the most common negatives associated with microwave cooking (Martin, 1988). Packaging techniques which enhance heating to cause dehydration and browning on the surface of products during microwave cooking, are currently being utilized. Energy absorbing packaging materials, called susceptors, utilize controlled thicknesses of metal, vacuum deposited onto paper, paperboard or film carriers. The metal droplets absorb microwave energy and generate localized heat (Perry, 1986; Dilberakis, 1987; Martin, 1988). Temperatures on the surface of the susceptor have been observed in excess of 200°C (Rosenkranz, 1987; Denford, 1988; Labuza, 1988). At these temperatures (>200°C), several safety concerns have been declared including increased migration of package components into the food product being heated (Bishop, 1987; Denford, 1988; Labuza, 1988; Mitchell, 1988; Rice, 1988b). In one study

of a tray-type susceptor (typical for pizza products) it was found that some species migrated close to 100% after 5 - 6 min of microwaving (Mitchell, 1988). It is speculated also, that formation of toxic components such as N-nitrosamines, may result from cooking of bacon in the microwave in contact with susceptors, because of the high temperatures associated with susceptor cooking.

Over the past two decades, the amount of N-nitrosamines in foods have come under close scrutiny due to their potential carcinogenic activity in animals. Many N-nitroso compounds have been shown to induce carcinogenic response in a variety of test animals (Hotchkiss, 1987b). N-Nitrosamines have been discovered in a number of food products, especially cured meats, e.g. bacon (Hotchkiss, 1987b). The formation of N-nitroso compounds in cooked bacon is affected by many factors such as: cooking methods (Pensabene et al., 1974; Bharucha et al., 1979), cooking time and temperature (Pensabene et al., 1974; Wasserman et al., 1978), lean-to-adipose tissue ratio (Fiddle et al., 1974), lipophilic inhibitors (Sen et al., 1976; Fiddler et al., 1978), storage period (Pensabene et al., 1980), concentrations of residual nitrite (Sen et al., 1974), thickness of slices (Theiler, al., 1981) and smoking (Theiler et al., 1984).

Use of susceptors in microwave "in-package" cooking may increase the temperature at the surface of the bacon sample while reducing cooking time (compared to frying), both of

factors which may affect the development of N-nitrosamines.

The objectives of this study were:

1. To measure the temperature of the product and package surface as a function of microwave cooking in susceptor and conventional (transparent) packages.
2. To develop a correlation between product temperature and formation of N-nitrosamines in microwave package cooking and frying.
3. To determine the effect of residual nitrite on the development of N-nitrosamines as a result of frying and microwave cooking.

LITERATURE REVIEW

A Trend to Microwave Foods

Social and demographic changes in the United State have created a significant increase in the demand for microwavable foods (Wright, 1983). The total number of households in the U. S. is increasing rapidly, while the number of persons per household is decreasing. In addition, more than half of all women between the age of 18 and 50 are in the work force (Martin, 1988). Thus, there is less time for preparation of the traditional meal and more disposable income. Convenience becomes a necessity in meal preparation, due to the lack of desire and/or time to cook (Wright, 1983; Martin, 1988).

Changes in lifestyle and user demographics have combined to create an explosion in the marketplace for microwavable foods (Sheridan, 1987). Convenience has been the key element in the success of microwavable foods (Martin, 1988). With approximately 80% penetration, the microwave oven has become one of the more common appliances in the U.S. household. About $3.4 billion will be spent in the microwavable food market segment by 1991 (Diberakis, 1987; Huang, 1987). Forty three million servings of food are prepared in the microwave daily, and there has been a 91% increase in microwave oven ownership among young singles and retired people (Sheridan, 1987). Typically, microwave oven owners are younger people between the age of 25 and 35 with total annual incomes over $30,000

(Martin, 1988). These people want the total convenience of opening a packaged frozen dinner, putting it directly in the microwave oven, eating it at the table and then throwing it away (Martin, 1988).

It is not surprising that the popularity of microwave ovens and higher quality convenience foods has triggered an expanding demand for microwavable products (Wright, 1983). Food processors have also accelerated the usage of microwave ovens by designing products and packages that use microwave heating/cooking to provide high quality products (Perry, 1986). Since there are unlimited opportunities to differentiate food products using current microwave food packaging, food manufacturers are designing convenient, high quality and time efficiency microwavable foods (Sheridan, 1987).

Microwavable foods have many convenience features (Table 1).

Table 1. Satisfactory Features of Microwave Cooked Food

Convenient	Easy
Heatable	No mess
Reheatable	No fuss
Saves time	Quick
Simple	Innovative

The major advantages of microwave heating versus conventional cooking are: speed, food prepared in the microwave ovens usually take only one quarter as much time, or less required, compared to conventional heating; uniformity of heating, since microwave energy penetrates food more efficiently than by conduction in the conventional oven, temperature distribution is more uniform and overheating may be avoided; high product quality, quality can be improved by reducing or eliminating case hardening; selective heating, microwave couples with selective materials which are absorptive of the energy, and which can lead to a greater heating efficiency in multicomponent food systems (Schiffmann, 1986).

Change in lifestyles is the primary factor in the U.S. behind the success of microwavable foods (Martin, 1988). In the future, there will be more single households, and less time to cook and prepare meals (Sheridan, 1987). Many more products will be packaged for microwave preparation because consumers appear to want them. Food processors and packagers are actively striving to participate in microwave market expansion. In addition, new microwave packaging technologies are emerging to provide better service, and more convenience features to satisfy the demand (Beck, 1989).

Mechanism of Microwave Heating

Microwave heating of foods is accomplished by directly transferring energy into foods using high frequency radiation.

The frequency agitates dipole molecules within products, and gives up energy as a result of temperature increase through molecule friction (Mudgett, 1986; Ohlsson, 1986; Diberakis et al., 1987). The most common dipole component in foods is water. Many foods contain large amounts of water, between 50 - 90 % (Ohlsson, 1986). Because of the dipole character of water, the microwave essentially heats products by activating the electrical charges of water molecules. These charges are arranged asymmetrically which is particularly active shape in a microwave field (Sacharow, 1988). Consequently, food products exhibit heating because of the excitation of water due to oscillation within the microwave field (Perry, 1986). Accordingly, microwaves are able to heat food without heating the surrounding environment as in a conventional oven (Bohrer, 1987; Sacharow, 1988).

In a microwave oven, the magnetron sends out electromagnetic waves into the oven cavity, which then bounce around the oven cavity until they are absorbed. The frequency inside the oven cavity results in an oscillating electrical and magnetic field. The electrical and magnetic fields coexist and are oriented in perpendicular planes. These fields are constantly changing, but there are certain configurations of energy that exist at any single point in time (Perry, 1986). Thus, materials with molecular dipoles, mobile ions or mobile electrons are affected by the electric field. The dipoles and ions try to realign themselves to the rapidly changing

electrical field, the motion created by these molecules causes the heating observed in a microwave oven (Perry, 1986).

There are two main mechanisms, by which microwaves produce heat within a microwave-irradiated product. These are "molecule-friction" resulting from the dipole rotation in a polar solvent and conductive migration of dissolved ions (Mudgett, 1986). The predominant heating mechanism is due to the rotation of dipole molecules as illustrated in Figure 1. In food systems, there are many dipole molecules, though water being the most common dipole material. Under normal conditions, polar molecules are randomly oriented. In the presence of an electric field, the polar molecules line up with the field. As the magnetron generates an oscillating magnetic and electric field (Perry, 1986), molecules will align themselves with the changing electric field. Heat is generated as a result of friction due to the rotation of molecules (Decareau, et al., 1987).

Heat is also produced due to the occurrence of ionic polarization. Conductive migration of ions in solution is induced in response to an electric field (Mudgett, 1986; Derakis et al., 1987). The moving ions contribute to the kinetic energy by means of the interaction with the electric field, this energy is then converted to heat when the ions collide with others (Fig. 2) (Decareau, et al., 1987). Though, heat is created predominately through the rotation of dipole molecules, intensified heating will occur if ionic materials

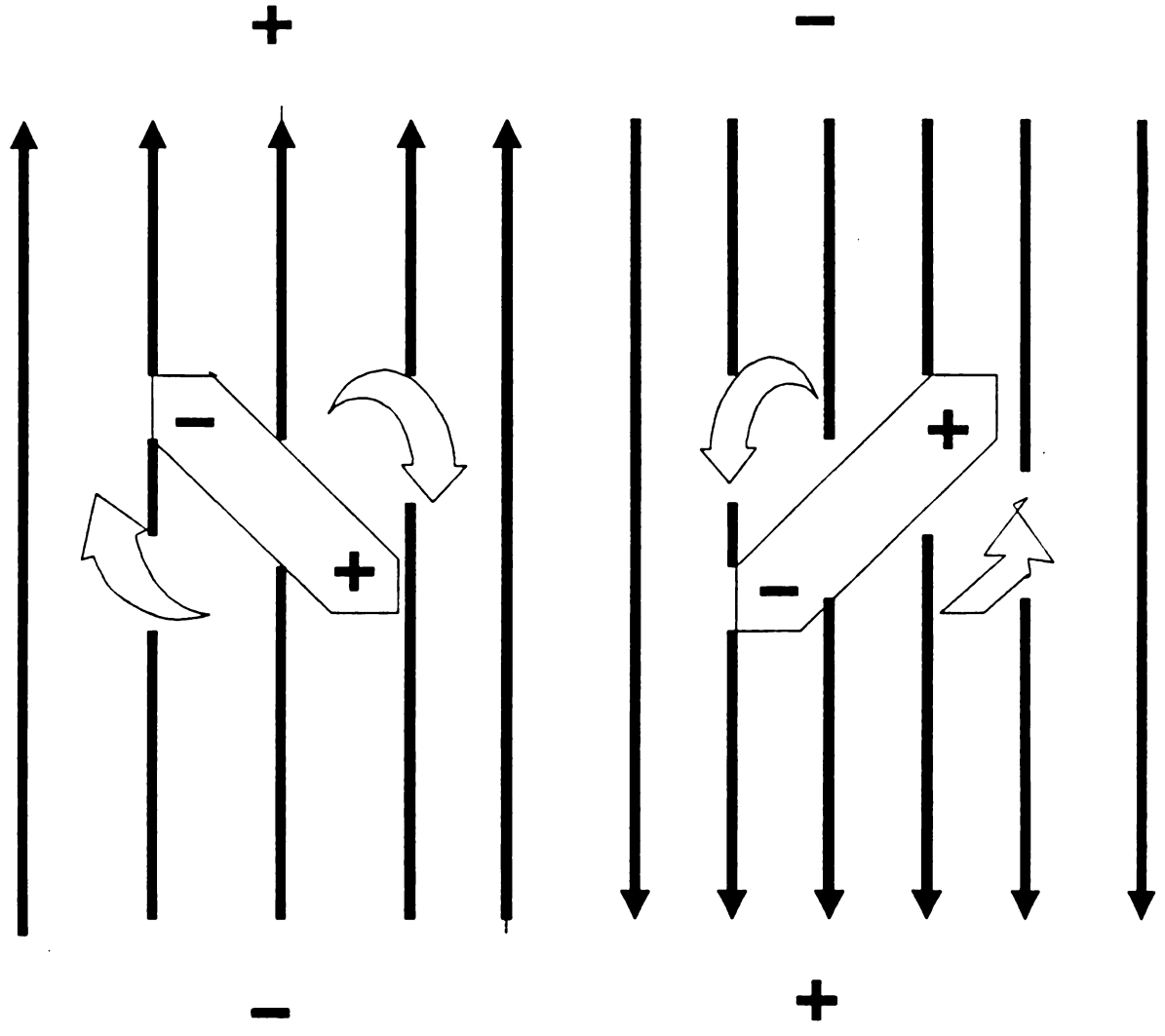


Fig. 1. Rotation of dipole molecules due to changing field (Decareau, 1987).

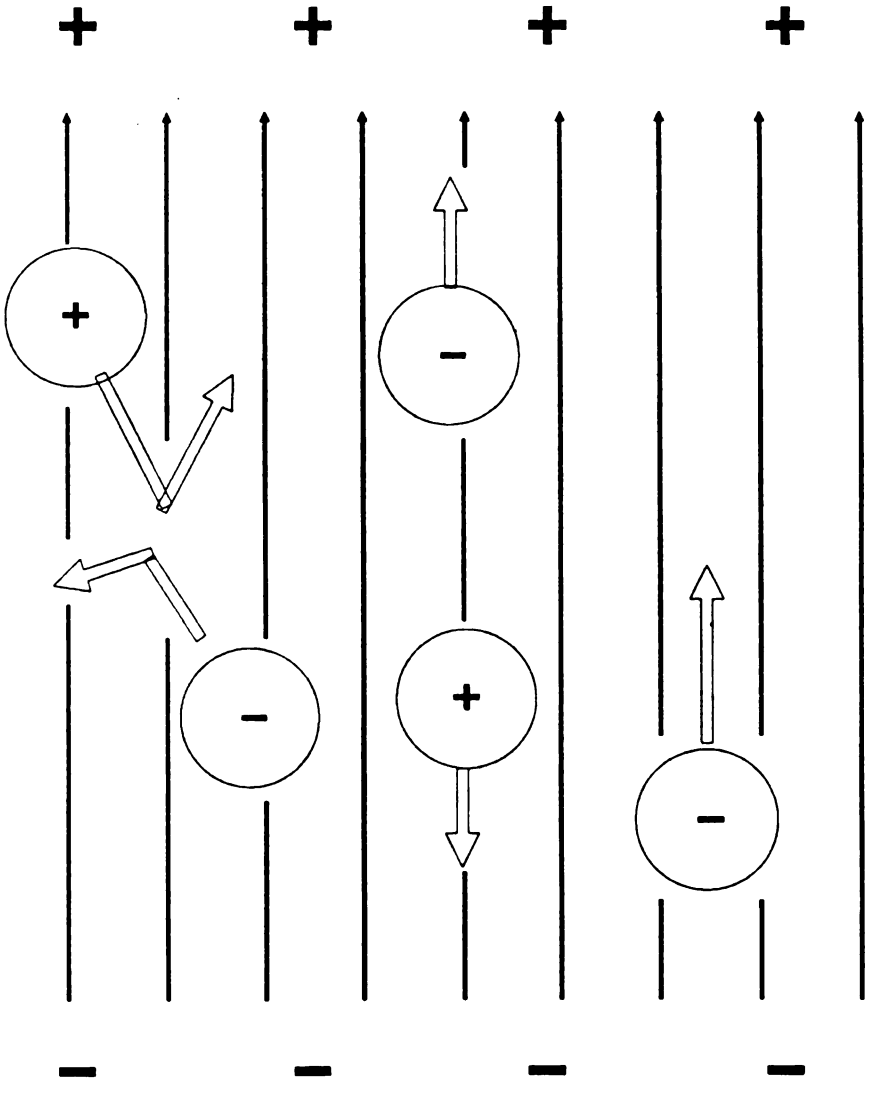


Fig. 2. Heat generated by collision of moving ions in the electric field (Decareau, 1987).

(salts) are dissolved in the aqueous phase (Perry, 1986).

Cooking in A Microwave Oven

Conventional ovens heat foods from outside in, the same is true in microwave ovens (Perry, 1986; Martin, 1988). An advantage of the microwave cook is that penetration depth is much greater than that experienced in conventional ovens (Perry, 1986). The essential differences between microwave and conventional cooking is illustrated in Table 2 (Bohrer, 1987). In conventional ovens, heated air in the oven gradually increases the temperature of food, which dries food out and aids in browning, crisping and driving moisture away from the surface (Martin, 1988). One further advantage of cooking in a conventional oven is the capability of preparing several food items together (Mudgett, 1989). Microwave food processes involve transferring energy through the surface, and reaching the interior of food, instead of by conduction (Perry, 1986; Martin, 1988).

Typically, the microwave thermal process provides deeper energy penetration, and heats food more efficiently. Mudgett (1988) demonstrated that conventional heating methods of transfer thermal energy from the product surface to the center are 10 - 20 times more slower, compared to microwave cooking. However, lack of hot air associated reduces browning and crisping, because there is little moisture evaporation at the surface of the food during heating (Martin, 1988). A

Table 2. The Fundamental Differences between Conventional and Microwave Cooking.

Conventional Oven	Microwave Oven
Hot air convection	Polar molecules excitation
Air hotter than food	Air cooler than food
Heat conducted from surface	Microwaves penetrate food
Conduction differences relatively small	Absorption differences relatively large
Surface dehydration before interior	Surface and interior dehydrate
Surface browning before bulk dehydration	Surface browning after bulk dehydration

Bohrer, 1987.

shortcoming of the microwave oven is its inability to cause browning and crisping (Perry, 1986). The lack of browning and crisping is attributed to low surrounding air temperature at the food surface, and high humidity conditions (Sacharow, 1988). During the processing of biological materials at medium to high temperatures, extensive mass (specifically moisture) will be transferred from center to surface, and some of it may be temperature-dependent (Ofoli, 1986). Cooling losses due to moisture evaporation at the surface is a major negative factor effecting crisping and browning (Mudgett, 1989).

Parameters Affecting Microwave Heating

Product size and shape, water, salt and fat content of the food, as well as product density and depth in the container influence the efficiency of coupling the food to the microwave environment (Drennan, 1987). Water content is usually the major attribute which influences the absorption of microwave energy. The more free water present, the higher is its dielectric loss factor, the more efficient is the heating (Schiffmann, 1986). Geometric properties also play an important role in microwaving. The more regular the shape of the food product, the more uniform heating can be obtained (Schiffmann, 1986). Sharp edges and corners tend to overheat, and sometimes cause arcing (Martin, 1988). When microwave energy passes through a curved interface, such as food with cylindrical or spherical geometry, the microwaves will

concentrate toward the center of the food, just as light does in a lens. This results in a high temperature in the center of the food (Ohlsson, 1986). The fat content also affects heating efficiency during microwaving. Microwave energy absorption by fat or cooking oil is very slow compared to water. However, fat is still heated to a high temperature (in excess of 100°C), and sometimes can be overheated due to a lower specific heat (Ohlsson, 1986). The specific heat is a physical index which describes the measurement of the temperature change in a material during thermal processing. When a certain amount of energy is provided to materials with the same weight, the one with lower specific heat will exhibit a greater temperature increase. Oil and fat are heated considerably faster than water because of their far lower specific heat, 2.0 joule /g °C of oil/fat to 4.2 of water (Schiffmann, 1986). Therefore, oil/fat displays twice the increase in temperature from the same quantity of energy, compared to water. Oil/fat demonstrates not only a greater temperature increase, but better energy maintenance. Materials having low boiling point, e.g. water, will consume energy to change their physical form (from liquid to vapor) at low temperature. However, many oil/fat systems do not boil until at least 200°C. More energy is maintained in an oil/fat product instead of lost due to vaporization. During microwaving, the energy absorbed by an oil/fat product may be transferred to the surroundings by conduction. Fats and oils

act thermodynamically as a "heat source", and release this energy to adjacent substances, because of the lower specific heat and higher boiling temperature (Connerton et al., 1989).

In designing microwave ovens, parameters such as frequency and power output, are primary factors affecting the heating mechanism. Fundamentally, frequency influences the penetration depth and heat response in objects (Schiffmann, 1986; Diberakis et al., 1987). Two frequencies are currently employed in microwave ovens, these are 915 and 2450 MHz. A frequency of 2450 MHz, which is utilized in household microwave ovens, is more effective in the generation of heat. However, 915 MHz produces a greater penetration depth (Decareau, 1985). The microwave power level also contributes to the time efficiency. The higher the power output, the faster will be the heating for a given mass (Schiffmann, 1986).

New Packages for Microwave Foods

Currently, several different packaging systems are employed for microwave food products. These include: transparent, absorbing (which are regarded as susceptors), shielding, and field modifying substances (Perry, 1986). Of these, transparent and absorbing based packages predominate. Susceptors were essentially developed to overcome limitations associated with crisping and browning. In order to achieve significant browning and crisping reactions, food surfaces

must be heated above 300°F (Anonymous, 1988; Sacharow, 1988). However, temperatures common to microwaving are less than 212°F (100°C), because of the boiling point of water (Ohlsson, 1986; Martin, 1988). Temperatures on the surface of susceptors are often in excess of 450°F (Breder, 1988; Labuza, 1988; Larson, 1988). At these temperatures, rapid dehydration on the surface of the food can be accomplished, resulting in crisping and browning.

Susceptors are constructed of a controlled thickness of metal which is usually vacuum deposited onto a plastic carrier, and adhesively laminated to a paper and/or paperboard backing (Huang, 1987; Martin, 1988). The metal layer provides the heating response (Diberakis, 1987). Molecules in the metal absorb the microwave energy and become agitated. The energy absorbed by the susceptor becomes a source of radiant heat, which reemits to the food surface and promotes crisping and/or browning (Diberakis, 1987; Huang, 1987). The plastic carrier functions as a support surface for the metallization, and protects the metal. The film also provides an approval food contact surface (Diberakis, 1987). In susceptors, crystallized PET (polyethylene terephthalate) is utilized predominately as the film substrate (Sheridan, 1987). Paper or paperboard substrates contribute dimensional stability during microwaving (Sacharow, 1988).

Susceptors increase the heat to the food by converting microwave energy, to a secondary heat source (Diberakis,

1987). A susceptor is usually attached to the cooking vessel in an area where crisping, browning and heat are demanded (Martin, 1988). Aluminum has been the prevalent metal used, because its molecular composition allows it to be heated to very high temperatures and the metallizing technology has been readily available (Martin, 1988). The key to successful heat generation using susceptors is the deposition of tiny droplets of metal onto the substrate surface. The frequency absorbing material must be isolated from one another on the surface of the film. If the film was completely metallized, the metal would act as a microwave shield, and no heat would be produced (Labuza, 1988). In 1986, approximately 83 million square feet of susceptor materials were consumed in frozen foods such as pizza, fries and waffles (Rosenkranz et al., 1987). In general, packaging of microwave foods must be tailored to the surface browning/crisping, interior cooking, and flavor/aroma needs of the particular food item under consideration (Huang, 1988).

Safety Concerns of Heat Enhancing Materials

Safety concerns have been raised relative to the high temperature process associated with susceptor based packages. Rapid microwave heating rates may cause rupture, overexpansion, or explosion of bakery products such as jelly doughnuts (Mudgett, 1989). Also, of particular concern is the potential migration of packaging components into food. In

addition, at temperatures in excess of 450°F (+200°C), physico-chemical changes in the food may occur, and result in the formation of hazardous compounds. These hazardous substances include N-nitrosamines in bacon and mutagenic imidazoquinoline compounds in cooked meat (Bharucha et al., 1979; Bishop et al., 1982; Bjeldanes et al., 1983; Bieber, et al., 1984; Felton et al., 1984; Startin et al., 1985).

Migration

Migration of low-molecular-weight substances from packaging materials into foodstuffs is influenced by the properties of the food components and interaction between the package and food. Migration rates can be affected by many properties of the food, including the fat content, pH value and alcohol content (Bieber et al., 1984). The mass transport process depends on concentration of migrants, exposure time, system temperature, product affinity to migrants, i.e. solubility and contact surface area (Bieber et al., 1984).

Heat intensifies the migration problem. At high temperatures, breakdown of packaging materials may occur, and result in acceleration in the migration rate. Moreover, increased migration not only increases the toxicological significance, but also decreases product quality (Bieber et al, 1984). Enhanced migration is induced when using susceptors to provide the localized heating of food. At temperatures of 400-500°F, migration of polymer oligomers and additives,

polymer decomposition products, adhesives and components of adhesives may occur (Breder, 1988). Bishop (1987) illustrated that for food being heated in plastic containers plasticizers were released from the plastic into the food. The heating of oils or fatty-type foods with microwave energy increases the rate of migration of plasticizers out of plastic film wraps. Increased temperatures and aging of polymer resins also tend to enhance not only the migration of plasticizers, but the migration of monomers and other additives (Bishop, 1987).

Typically, the highest temperature specified for any packaging film by the Food and Drug Administration (FDA) is 275°F, however, microwave susceptors often heat at 400-500°F (Labuza, 1988; Mitchell, 1988). At temperatures in excess of 500°F, there is no functional barrier between the food and the susceptor packaging components (Mitchell, 1988).

A close examination of microwaved packages shows "clear evidence" of cracking and melting of the polyester film and browning of the paperboard (Mitchell, 1988). A simple dye test revealed that the cracking went below the surface and penetrated into the paper and adhesives layers. The migration of PET residues into corn oil illustrated that extent of migration increased with the heat experienced. With the tray-type susceptors used in pizza products, migration levels were doubled compared to virgin PET after 5 to 6 min of microwaving (Mitchell, 1988).

Recently, the FDA has expressed safety concerns on the

degradation problem with adhesives, polymers, paper and other components of susceptor microwave packages. The major concerns of the FDA were as follows (Hollified, 1988):

1. Breakdown of packaging material (packaging materials begin to crack during microwaving).
2. Chemical migration from the food contact surface increases during microwaving.
3. Does food product contact layer provides sufficient protection of the food from potential migrants?

At the extremely high temperatures (450 - 500°F) generated in susceptors by microwave heating, the possibility of migration, ignition of oils and fats, and other possible mishaps have sparked an industry-wide clamor for government-approved testing procedures for microwave foods/packaging (Larson, 1988).

N-Nitrosamines in Foods

For the past two decades, the concentration in food of N-nitroso compounds has been monitored carefully, because of their potential carcinogenic activities in animals. The acute toxicity of N-nitrosodimethylamine was investigated in animals in the 1930's. Severe liver failure was found, and the more acutely poisoned individuals died of liver necrosis approximately 7 weeks after exposure (Hotchkiss, 1987b). More than 80% of the 300 N-nitroso compounds tested have been found to be carcinogenic in relation to one or more animal species (Magee et al., 1976). N-Nitrosamines have been observed in a number of food products, including cured meat (especially cooked bacon), dairy products, tobacco and alcoholic beverages (Table 3) (Hotchkiss, 1987b).

The N-Nitrosamines in bacon are primarily N-nitrosodimethlyamine (NDMA) and N-nitrosopyrrolidine (NPYR). Many factors affect the concentration of these compounds in cooked bacon, including cooking method, cooking temperature and time, residual nitrite, lipophilic inhibitors, lean-to-adipose ratio, age of pork bellies, thickness of slices, fatty acid composition, and smoking process (Skrypec et al., 1985).

Chemistry of Formation

N-Nitrosamines are primarily formed from the reaction between secondary amines and nitrosating agents, but also can be produced from primary, tertiary and polyamines (Fig. 3)

Table 3. Survey of Foods for N-Nitrosamines.

Survey and Food	No. positive /No. analyzed	N-Nitroso compounds	Range (μg/kg)
Havery et al. (1976), in U.S.			
Fried bacon	22/22	NPYR	7-139
Variety meats	1/ 6	NPYR	6-NR[a]
Gough et al. (1977), in U.K.			
Fried bacon	33/56	NDMA, NPYR	ND[b]-200
Canned, cured meats	9/34	NDMA	ND- 10
Fish	28/112	NDMA	ND- 10
Cheese	10/58	NDMA	ND- 15
Cured meats	25/64	NDMA, NDEA	ND- 8.6
Kawabata et al. (1980), in Japan			
Salt-fermented vegetables	6/49	NDMA, NPYR	ND- 5
Beer	27/29	NDMA, NPYR	ND- 5
Spiegelhalder et al. (1980), in Germany			
Meat product	127/395	NDMA, NPYR	0.5- >5
Cheese	49/209	NDMA	0.5- 5
Beer	142/215	NPYR	ND- 68
Sen et al. (1980), in Canada			
Cured meats	77/118	NDMA, NDEA NPYR, NPIP	ND- 55
Alcoholic beverage	23/50	NDMA	ND- 4.9
Osterdahl (1988), in Sweden			
Fried bacon	68/68	NDMA, NPYR	1.7- 7.6
Smoked pork, fried	20/21	NDMA, NPYR	0.9- 4.0
Smoked fish	55/61	NDMA	ND- 1.3
Beer	153/258	NDMA	ND- 0.3
Spice mixture	4/4	NDMA, NPYR NPIP	0.7- 1.5

a. NR, not reported
b. ND, not detected

Hotchkiss, 1987b; Osterdahl, 1988

(Gray et al., 1979). In an acidic environment, nitrite is converted to nitrous acid (HONO) which then reacts with primary, secondary and tertiary amines to form N-nitrosamines (Lijinsky et al., 1972).

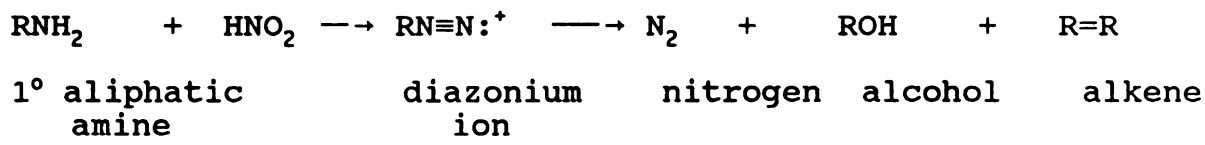
When secondary amines are treated with nitrous acid, nitrosation occurs and stable N-nitrosamines are formed (Fig. 3). The first step in the nitrosation of a tertiary amine is similar to the reaction path for primary and secondary amines. The reaction is accomplished when the unshared electron pairs with the unprotonated amines and reacts with a nitrosating agent (nitrous anhydride), forming the nitrosoammonium ion (Fig. 3). These ions undergo cis-elimination of the nitroxyl ion to form immonium ions which are hydrolyzed to carboxyl ions. Thus, the secondary amine is nitrosated to the corresponding N-nitrosamines (Fiddler, et al., 1972).

Formation of NDMA and NPYR in Bacon

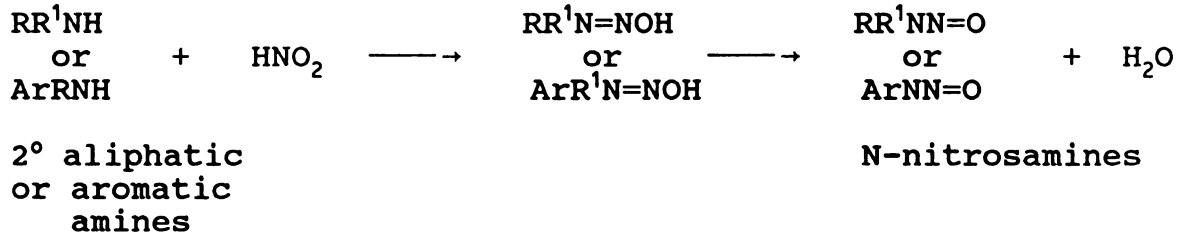
N-nitrosamines have been frequently detected in cooked bacon, including NDMA, NPYR, N-nitrosodiethylamine (NDEA), NPYR and N-nitrosopiperidine (NPIP). However, the major N-nitrosamine present in bacon is NPYR.

NDMA has been shown to be more carcinogenic than NPYR (Magee et al., 1976)., and is found consistently in bacon. Up to 80% of NDMA is lost in the vapor during preparation (Gray et al., 1977). The formation of NDMA in bacon is not clearly understood. However, results from several model studies have

Primary Amines



Secondary Amines



Tertiary Amines

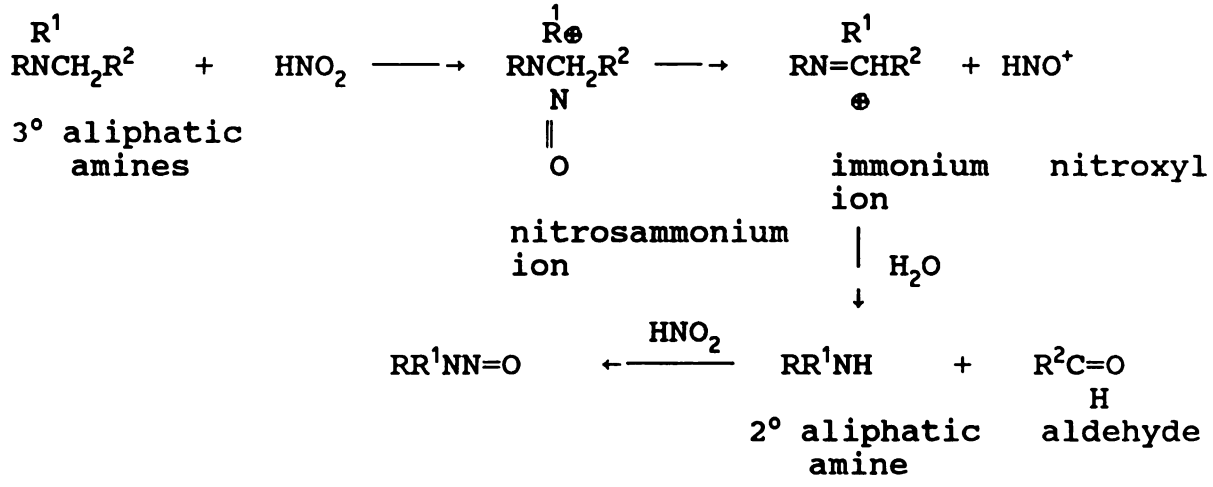


Fig. 3. Reactions of primary, secondary and tertiary amines with nitrous acid (Lee, 1981).

indicated that various compounds, including lecithin, sarcosine, and tri- and di-methylamines can form NDMA on reaction with nitrite (Fiddler et al., 1972). Compounds containing sarcosine and choline were proposed to be the most probable amine precursors of NDMA (Gray, et al., 1978). However, the concentration of sarcosine and choline in pork bellies has not been estimated.

The consistent occurrence of NPYR in fried bacon has been reported in many studies (Havery et al., 1976; Gough et al., 1977; Spiegelhalder et al., 1980; Sen et al., 1980; Osterdahl, 1988). Studies of food systems have implicated several compounds including proline (PRO), pyrrolidine (PYR), collagen, putrescine and spermidine as potential precursors of NPYR, with PRO as the most likely candidate (Gray, 1976). Although the formation of NPYR from PRO has not thoroughly understood, two pathways have been proposed (Gray, 1976; Bharucha et al., 1979). The possible pathways for NPYR formation in bacon are illustrated in figure 4.: in the first pathway, PRO is initially nitrosated to N-nitrosoproline (NPRO), NPRO then undergoes thermal decarboxylation to yield NPYR. In the second pathway, the initial thermal decarboxylation of PRO to pyrrolidine (PYR), is followed by nitrosation to NPYR. Since decarboxylation of NPRO to NPYR occurs at a much lower temperature than from PRO to PYR, the initial pathway seems the more favorable (Bharucha et al., 1979; Lee et al., 1983b).

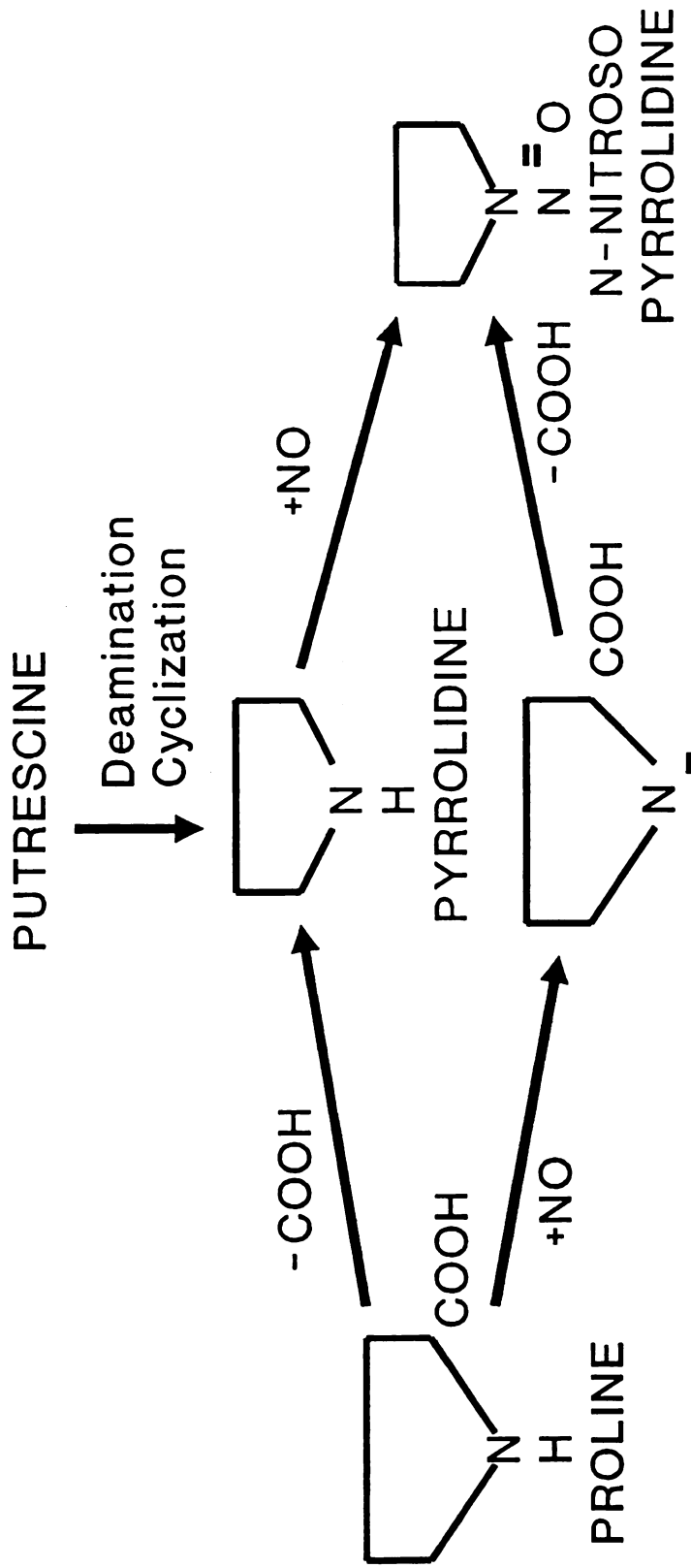


Fig. 4. Possible pathway for the foramtion of NPYR in bacon.

(Lee, 1981)

Toxicological Concerns of N-Nitrosamines

About 300 N-nitroso compounds have been tested, and more than 80% have been shown to have carcinogenic activity in laboratory animals (National Academy of Science, 1981). However, the major safety concern of N-nitroso compounds to human is the chronic exposure, instead of the instantaneous response. The environment/food exposure rates of N-nitroso compounds is less than the amount required to produce an acute toxic effect in the short term (Hotchkiss, 1987b). However, the concentrations are high enough to cause tumors in experimental animals through long term exposure (Magee et al., 1976; Sen et al., 1980).

It is generally believed that N-nitroso compounds must be metabolically activated to express carcinogenic activity (Archer, 1982). The metabolic activation of N-nitrosamines involves enzymatic hydroxylation of an α carbon atom to the N-nitrosoamine nitrogen. Therefore, the alpha position of the N-nitrosamines has been associated with the carcinogenic action of these compounds (Magee et al., 1976).

Animal experiments indicate that the effect of the N-nitrosamine in chronic exposure is cause liver necrosis and/or hemorrhaging, and the carcinogenesis of N-nitrosamines has been shown to be organ specific (Hotchkiss, 1987b). N-Nitrosodimethylamine and N-nitrosopyrrolidine, the most widely occurring N-nitrosamines in foods, cause mostly liver tumors and occasionally kidney tumors in animals (Magee et al.,

1976).

Factors Affecting N-Nitrosamine Formation

Factors influencing the formation of N-nitrosamines in bacon are well documented (Skrypec et al., 1985). Studies outlining contribution of the different factors are discussed below.

Cooking Methods

Pan frying has been shown to produce greater amounts of N-nitrosamine in bacon, compared to broiling and grilling (Pensabene et al., 1974; Wasserman et al., 1978; Bharucha et al., 1979). A high internal temperature contributes to greater N-nitrosamines in cooked bacon (Wasserman et al., 1978). Similarly, draining of the cook-out-fat during grilling lowered the internal temperatures, and resulted in less N-nitrosamine formation in bacon (Bharucha et al., 1979).

Heating Temperature and Time

Temperature and time of cooking play an important role in the formation of N-nitrosamine. Only 10% conversion to NPYR was found at 100°C compared to that produced at 180°C (Pensabene et al., 1974). The maximum NPYR formation in bacon was observed when bacon was fried at 182°C (350°F) for 12 min (Bharucha et al., 1979). Generally, the N-nitrosamine content in cooked bacon begins to increase after 4 min, and reaches

maximum at about 12 min, and then declines due to volatilization (Pensabene et al., 1974). Pensabene et al. (1974) also found that the optimum temperature for N-nitrosamine formation was 185°C, which is near normal pan frying temperatures. During frying, the internal temperature of the lean tissue reached a maximum of 110°C (Coleman, 1978). However, after the majority of the water has vaporized, the temperature of rashers can reach 180°C (Coleman, 1978). Therefore, it was proposed that the majority of N-nitrosamines are produced after the bulk of water in bacon has volatilized (Pensabene et al., 1974; Coleman, 1978; Bharucha et al., 1979).

Concentration of Residual Nitrite

The role of nitrite in cured meat functions to produce the characteristic cured meat color, texture and flavor, and inhibits the outgrowth of Clostridium botulinum and acts as an antioxidant (Hotchkiss et al., 1987a). However, nitrite is also the primary reagent involved in N-nitrosamine formation.

The rate of nitrosation is directly proportional to the square of the nitrite concentration (Mirvish, 1975). Sen et al. (1974) reported that NDMA and NPYR concentrations in fried bacon were highly correlated with the initial nitrite level. Robach et al. (1980) reported that a reduction in the initial sodium nitrite level from 120 to 80 mg/kg, resulted in significantly lower NPYR concentrations in bacon. Therefore,

the lower the residual nitrite the less is the probability of N-nitrosamine formation (Dudley, 1979).

Storage of Pork Bellies

The length of storage of pork bellies prior to processing affects the final N-nitrosamine formation in cooked bacon. Greater concentrations of NPYR were reported in fried bacon, when aged bellies were used in comparison with fresh pork bellies (Pensabene et al., 1980). The free proline content has been shown to increase by 50% in whole bellies, and approximately 90% in the adipose tissue after one week of storage at 2°C (Gray et al., 1977). The increase in free amines during aging due to proteolysis, therefore, produces greater NPYR concentrations after frying (Pensabene et al., 1980).

Thickness of Slices

Since the formation of N-nitrosamines is related to product cooking temperature of frying, the thickness of the slices may affect NPYR formation. Theiler et al. (1981a) illustrated that as thickness increased, NPYR concentrations decreased. Thicker slices do not allow as high an internal temperature to be reached during frying, and results in decreased quantities of N-nitrosamines.

Lipophilic Inhibitor

Bharucha et al. (1979) observed that formation of N-nitrosamines during the frying of bacon occurred almost entirely in the fat phase by a free radical mechanism rather than an ionic mechanism. A compound which can successfully compete with a secondary amine for a nitrosating species results in the reduction of N-nitrosamines formation, is regarded as a blocking agent (Gray et al., 1975). Lipophilic inhibitors, function as blocking agents, and serve to trap NO radicals in the fat phase, and block the nitrosation reaction of amines (Bharucha et al., 1979). Alpha-tocopherol and ascorbic acid derivatives have been shown to inhibit N-nitrosamine formation by lipophilic reduction (Sen et al., 1976; Fiddler et al., 1978; Bharucha et al., 1980).

Reduction in N-nitrosamines by ascorbic acid and its isomer, erythorbic acid (d-erythro-ascorbic acid), was first discussed by Mirvish et al. (1972). Inhibition involves oxidation of the vitamin C to dehydroascorbic acid, and the reduction of nitrous acid to nitric oxide (Mirvish et al., 1972). Another ascorbate compound, ascorbyl palmitate, also reduces N-nitrosamine formation in bacon by 70-90%, when 500 - 1000 mg/kg is added in the brine (Bharucha et al., 1980). Although, reduction in N-nitrosamine has been accomplished using these compounds, ascorbates are not completely successful in inhibiting NPYR formation, because of their limited solubility in adipose tissue.

Alpha-tocopherol has been reported to inhibit NPYR formation by 90% when used at a level of 500 mg/kg (Gray et al., 1982). This lipophilic antioxidant reacts with nitrosating agents in a manner similar to ascorbates. However, α-tocopherol has the added benefit of not undergoing C-nitrosation (Mergens et al., 1979). α-Tocopherol reduces nitrogen oxide to form nitric oxide, while it undergoes oxidation to quinone (Hotchkiss, 1987b). An optimum inhibition of nitrosation was obtained using 500 mg/kg of α-tocopherol and 550 mg/kg of ascorbate (Gray et al., 1982). The combination of α-tocopherol and ascorbate more effectively inhibited N-nitrosamine formation than either compound alone. This is due to the fact that ascorbate inhibits N-nitrosamine formation in the aqueous phase, while α-tocopherol acts in the lipid phase.

Fatty Acid Composition of Adipose Tissue

Formation of the majority of N-nitrosamines appears to be associated primarily with the adipose tissue during frying (Pensabene et al., 1974; Bharucha et al., 1979). Increased NDMA and NPYR formation in fried bacon were observed in association with elevated amounts of unsaturated fatty acids in the pork bellies (Skrypec et al., 1985). The fat portion in bacon yields 12 times more NPYR, and 6 times more NDMA than the lean portion during frying (Mottram et al., 1977). One possible mechanism was illustrated by Walter et al. (1979).

Unsaturated fatty acids were shown to act as transnitrosating agents in the fat phase. The study demonstrated that unsaturated fatty acids are capable of interacting with nitric oxide (nitrite) to form α-nitroso-nitrite esters with unsaturated double bonds. The α-nitroso-nitrite esters of unsaturated triglycerides can then transnitrosate to secondary amines to produce N-nitrosamines in the adipose tissue.

Smoke

Cured meats are smoked in order to enhance preservation, and to develop characteristic flavor and color. Pensabene et al. (1983) indicated that some nitrosating species may be generated by nitrogen oxides during the smoking process. Some components of wood smoke, including formaldehydes and nitrophenols, could possibly be involved in the N-nitrosation reaction (Mandagere, 1986). However, Bharucha et al. (1980) reported that smoked bacon contained less NPYR and/or NDMA compared to unsmoked samples, presumably due to a lowering of pH by the acidic smoke ingredients. The effects of smoking seem to be due to a combination of lowering the pH, and direct C-nitrosation of phenolic compounds.

METHODS AND MATERIALS

Susceptor Material

The susceptor material was obtained in sheet form (60.5 cm x 90.7 cm) from a material manufacturer. The susceptor material was constructed of metallized (aluminum) polyethylene terephthalate (PET), and adhesively laminated to paperboard. The PET surface is the food contact layer.

Preparation of Bacon Samples

Pork bellies were processed into bacon within 48 hr of slaughter in the Meat Laboratory, Michigan State University. Bellies were stitch pumped to 110% (wt/wt) of their green weight using a brine, to obtain a target concentration of 1.5% sodium chloride, 0.35% sodium tripolyphosphate, 0.5% sucrose, 120 mg/kg sodium nitrite, and 550 mg/kg sodium ascorbate. To study the effect of nitrite concentrations on the formation of N-nitrosamines, pork bellies were cured using the same brine as described previously, except bellies were pumped to obtain a target concentration of 200 mg/kg sodium nitrite instead of 120 mg/kg.

Pumped bellies were placed into polyethylene bags after stitch pumping, and the bellies allowed to equilibrate overnight at 2°C. Bellies were then transferred to an Elec-Trol laboratory smokehouse (Drying System Inc., Chicago, IL), and cooked at 58°C (dry bulb temperature) for 4 hr, followed

by three hours of cooking at 52°C (dry bulb temperature). The smoked bellies were transferred to a cooler (2°C) and stored overnight prior to slicing and packaging (Reddy et al., 1982).

After smoking, the slab bacon was sliced into 1/8 in (0.32 cm) thick slices. Bacon was placed onto polystyrene trays, deposited into polyethylene pouches, and then sealed under vacuum. The packaged samples were stored at 2°C for 7 days prior to cooking.

Two commercial slabs of bacon, purchased from a local retail store were also used in this study. Samples were sliced (0.32 cm thick), placed onto polystyrene trays and sealed under vacuum in polyethylene pouches immediately after purchase. The raw bacon was stored at a temperature of 2°C for one week prior to cooking.

Following cooking, bacon samples were stored in 18 oz polyethylene pouches (Whirl-Pak, NASCO, Ft. Atkinsos, WI). Cooked samples were deposited into separate pouches, closed and placed in a freezer at a temperature of -5°C prior to analyze the cooked bacon samples for N-nitrosamine content.

Determination of Residual Nitrite

Residual sodium nitrite in the raw bacon was determined using the AOAC procedure (AOAC, 1984). A 5 g finely comminuted sample was weighed and mixed with 40 ml (80°C) water to break up the lumps and then transferred into a 500 ml volumetric flask. To the mixture was added enough hot water to bring it

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to ca 300 ml. It was then placed in a steam bath for 2 hr.

After cooling to room temperature, the mixture was diluted to 500 ml using distilled water. The solution was filtered using Whatman No. 1 filter paper, and 25 ml of filtrate was transferred to a 50 ml volumetric flask. A 2.5 ml volume of sulfanilamide reagent was added to the filtrate, along with 2.5 ml of NED (N-(1-naphthyl) ethylenediamine) reagent after 5 min. The solution was diluted to 50 ml using distilled water, and the color was allowed to develop for 15 min.

Residual nitrite in the raw bacon was determined using a spectrophotometer (Spectronic 2000, Busch & Lomb, Rochester, N.Y.) to measure the absorbancy at 540 nm. A mixture of 45 ml distilled water, 2.5 ml sulfanilamide and 2.5 ml NED reagent was used as a blank to calibrate the optical response of nitrite.

A standard curve was used to determine nitrite present in the raw bacon. The amount of residual nitrite was based on comparison of the absorbancy at 540 nm to a standard curve. The standard curve was established using 1, 2, 5, 10, 20, 30 and 40 ml of a 1 μg/ml $NaNO_2$ solution.

The concentration of residual nitrite in the samples was calculated as:

$$\left[\begin{array}{c}\text{AMOUNT READ}\\ \text{FROM}\\ \text{STANDARD CURVE}\\ (\mu g)\end{array}\right] \times \left[\frac{\frac{500 \text{ ml Total}}{25 \text{ ml Aliquot}}}{5 \text{ g sample}}\right] = \begin{array}{c}\text{NITRITE}\\ (\text{mg/kg meat})\end{array}$$

Determination of Power Output of Microwave Oven

The power output of the microwave oven was calibrated prior to cooking the bacon. The power output was determined three times, and an average reported.

The measurement was accomplished at the maximum microwave power setting with a load of 2000±5 g of potable water. The microwave oven was clean and free from grease using paper towels to wipe the cavity wall prior to cooking, and then warmed up by placing a one liter beaker containing a liter of water in the cavity. The microwave oven was operated on full power (100%) for 10 min. The oven was then allowed to cool to room temperature, and the test repeated within 20 minutes of the oven shut off. Potable water (2000 g) was divided equally into two 1-liter beakers, the initial temperature of water was in the range 18 - 22°C (64.5 - 71.5°F). The water was stirred using a glass rod, and the initial temperature in each beaker was measured and recorded.

The beakers were placed in the center of the oven in contact with each other. The microwave oven was operated at full power for 2 min and 2 sec (to compensate for the filament

warm up time). The water in each beaker was stirred immediately after heating. The temperature was then measured and recorded in each of the beakers. This procedure was repeated two more times, each test was repeated within 20 min of shutoff of the last test.

To determine microwave power output in watts the following calibration was used as follows:

$$\text{POWER (Watt)} = 70 \left\{ \frac{\Delta T_1 + \Delta T_2}{2} \right\}$$

where,

$\Delta T_{1,2}$ are the temperature rise of the water in the beakers in degrees Celsius, which is determined by subtracting the initial water temperature from the final temperature.

Determination of the "Hot Spots" in the Microwave Oven

The susceptor microwave material functions by absorbing energy from the magnetron in the microwave oven. The energy inside the oven cavity is delivered in the form of an oscillating electrical and magnetic field. The field changes at any point in time, therefore, localized heating exists, which creates "Hot" and "Cold" spots (Perry, 1986). A spot in the path of the microwave field, where responding intense energy results in a high temperature, is characterized as a hot spot. Faster heating is obtained when samples are placed on the hot spot. Therefore, determination of hot spots is

necessary prior to all microwave induced thermal processes.

Susceptor sheets were used to determine the hot spots in the microwave oven (Amana Refrigeration Inc., model RS458P, 700 Watts, Amana, IA). A significant attribute of susceptors is their ability to absorb microwave energy and convert it into heat. In the construction of a susceptor, a controlled thickness of metal is deposited on a plastic carrier, and laminated to a paper/paperboard backing (Huang, 1987; Martin, 1988). Since the paper backing has only moderate thermal resistance, charred marks will be observed as a result of heat from the aluminum particles. These marks are characterized as the hot spots.

Determination of the hot spots was accomplished using a sheet of susceptor material cut to match the inside dimensions of the oven floor, and placed directly onto the floor of the oven cavity. The susceptor was then exposed to 5 min of heating at full power. The susceptor sheet was then inspected and the location of the hot spots were recorded.

Thermal Processing of Bacon

Bacon samples were prepared conventionally in a fry pan and in two different microwave packages, in order to determine the effect of cooking methods on the formation of N-nitrosamines. Frying was accomplished using a preheated teflon-coated electric pan at a thermostat setting of 171°C (340°F) for 3 min on each side (6 min total). Three slices of

bacon (80 - 100 g) were fried simultaneously, and a total of 15 slices of bacon were cooked using frying. After frying, the fried bacon samples were placed on paper towels to drain away the cook-out-fat.

Microwave "in-package" cooking methods were accomplished using the susceptor and microwave transparent materials in a microwave oven (Amana Refrigeration Inc., model RS458P, 700 watts, Amana, IA). The output power of the microwave oven was calibrated prior to use, and found to be 670 watts. Microwave transparent packages were commercial products, purchased from a retail store. The microwave transparent trays were made from CPET (allowed the temperature of use uo to 450°F), and had dimensions of 15.3 cm x 20.5 cm (W x L). Susceptors were obtained from a material supplier. The susceptor was used by cutting the susceptor sheets into the same dimensions as the bottom area of the transparent tray, and then placing the susceptor onto the bottom of the tray.

The bacon samples were cooked in the microwave oven at full power. Three slices of bacon (80 - 100 g) were used in both microwave cooking methods, 15 slices of bacon were used in each microwave cooking. Cook-out-fat was drained off on paper towels. Heating times were varied to achieve a specific "degree of doneness" compared to frying. Cooking times of 2, 3 and 4 min were used for transparent in-package cooking, and 2, 2.5 and 3 min for susceptor in-package cooking. Degree of doneness was based on the browning characteristic of the

cooked bacon and "same degree of doneness" determined using a small panel.

Determination of Material and Bacon Temperatures during Cooking Using Fluoroptic Probes and Thermocouples

A thermocouple system (Omega Engineering Inc., Gardiner, N.Y.) was used to measure the temperature of bacon during frying. Four probes were placed at the interface between the bacon sample and the frying pan. Temperature readings were recorded every 30 seconds.

For the bacon prepared in the microwave oven, temperatures on the interface of the packaging material and bacon surface were measured using a Luxtron 755 Multichannel Fluoroptic Thermometer (Luxtron, Mountain View, CA). Two MIW probes were placed onto the surface of the empty microwavable packaging material and/or interface between the samples and the packaging material. Temperatures were measured and recorded every 10 seconds using a data collection computer system.

Determination of N-Nitrosamines in Cooked Bacon

N-Nitrosamine concentrations in cooked bacon were quantitated using A Dry Column - Thermal Energy Analyzer method (AOAC, 1984).

All chemicals were analytical grade and used without further purification. Dichloromethane (CH_2Cl_2) was purchased

from Mallinckrodt Inc. (Paris, KY), and redistilled prior to use. N-Pentane was HPLC grade and also purchased from Mallinckrodt. N-Nitrosamine standards were purchased from Thermedics Inc., Woburn, MA.. Celite 545 (Fisher Scientific Co.) was prewashed with CH_2Cl_2 twice, and then dried in a oven (110°C) for 4 hr prior to use.

Celite (10 g) was mixed with 10 ml of 6N HCl, and poured into a chromatographic column. Bacon samples were blended in dry ice using a Waring Blender (model 33BL79, New Hartford, CT), and then held at room temperature until all dry ice evaporated. Ten grams of the bacon were transferred into a mortar, and 25 g of anhydrous sodium sulfate (Na_2SO_4) and 10 g Celite were added and mixed into the bacon. This mixture was then poured into the column. One ml of the internal standard, N-nitrosoazetidine (NAZET) (0.1 μg / ml CH_2Cl_2), and 30 g of Na_2SO_4 were deposited on top of the column.

Extraction was initiated by adding 100 ml of a N-pentane - dichloromethane mix (95 : 5). When the level of solvent dropped to the top of the Na_2SO_4, 125 ml CH_2Cl_2 was added to the column. The initial 85 ml of wash eluent was discarded, and the remaining eluent was collected into a 125 ml flask. The eluent was transferred into a Kuderna-Danish (K-D) flask (Knote Glass Co.) equipped with a concentrator tube which was attached to a 3-section Snyder distillation column (Knote Glass Co.). The eluent was concentrated to 4 ml using a steam bath, and then concentrated to 1 ml by flushing with nitrogen.

An aliquot (5 μl) of the concentrated extract was then injected into a GC-TEA (Varion Aerograph G.C. model 3700 interfaced with a TEA Model 502). A 2.7 m x 3.2 mm (ID) glass column (Supelco, Bellefonte, CA) packed with 15% Carbowax 20M-TPA was used to separate the N-nitroso compounds. Thermal conditions were set initially at 140°C, and programmed to 180°C at a rate of 7°C / min. The peak area of the extracted N-nitrosamines was calculated using a Hewlett Packard Model 3390A Integrator (Walnut Creek, CA).

The analytical efficiency was monitored by determining the recovery of the internal standard (NAZET). An aliquot of 5 μl (0.1 μg NA /ml) standard solution containing seven N-nitroso compounds, including N-nitrosodimethylamine (NDMA); N-nitrosodiethylamine (NDEA); N-nitrosodipropylamine (NDPA); N-nitrosodibutylamine (NDBA); N-nitrosopiperidine (NPIP); N-nitrosopyrrolidine (NPYR) and N-nitrosomorpholine (NMOR), was used for identification and quantitation of N-nitroso compounds in the cooked bacon. Specific N-nitrosamines were identified by comparing their retention time to those of the standard N-nitrosamines, and the amount of N-nitrosamines in the bacon was determined according to their relative response area.

The amount of N-nitrosamines formed in cooked bacon was calculated by:

$$\text{N-nitrosamine } (\mu g/kg) = \left(\frac{A_{sample}}{A_{standard}} \right) \times \left(\frac{\text{CONC. OF STANDARD}}{\text{wt of sample (g)}} \right) \times 1000$$

where, A_{sample}, represents the response area of N-nitrosamines in cooked bacon. $A_{standard}$, represents the response area of N-nitrosamines in the working standard.

STATISTICAL ANALYSIS

Correlation between formation of N-nitrosamine and cooking methods was determined by measuring the NPYR concentration versus Temperature-Time History of the different cooking methods. The Temperature-Time history was determined by integrating the area under the Temperature - Time profile.

Statistical comparison of N-nitrosamine data relative to the three cooking methods and residual nitrite levels was accomplished using the Bonferoni t-test (Gill, 1978).

RESULTS AND DISCUSSION

Temperature Profile of Microwave Cooked Bacon

A rapid increase in temperature on the surface of the empty susceptor was obtained during heating in a 700 Watt microwave oven. Temperatures in excess of 100°C (212°F) were observed within 30 sec. A final temperature of 220°C (430°F) was obtained on the susceptor material after microwave heating for 3.5 min (Fig. 5). Less heat was generated in the transparent package. An elevation in temperature was found on the surface of the empty transparent package during microwaving. However, no temperature greater than 110°C (230°F) was observed after 6 min of microwaving (Fig. 5). The susceptor material more efficiently converted microwave energy into heat than did the transparent package (Fig. 5).

Lower temperatures were obtained when the susceptor packages were loaded with bacon during heating. A decreased temperature was observed after cooking for 2 min (Fig. 6). The highest temperature recorded was 113°C (235°F). Higher temperatures were observed with increased cooking times. Final temperatures of 166°C and 211°C (331°F and 412°F) were obtained at 2.5 and 3 min of microwaving, respectively (Fig. 6). However, no temperature was observed greater than those found on the empty susceptor for the same time period (Fig. 6).


TEMPERATURE (C)

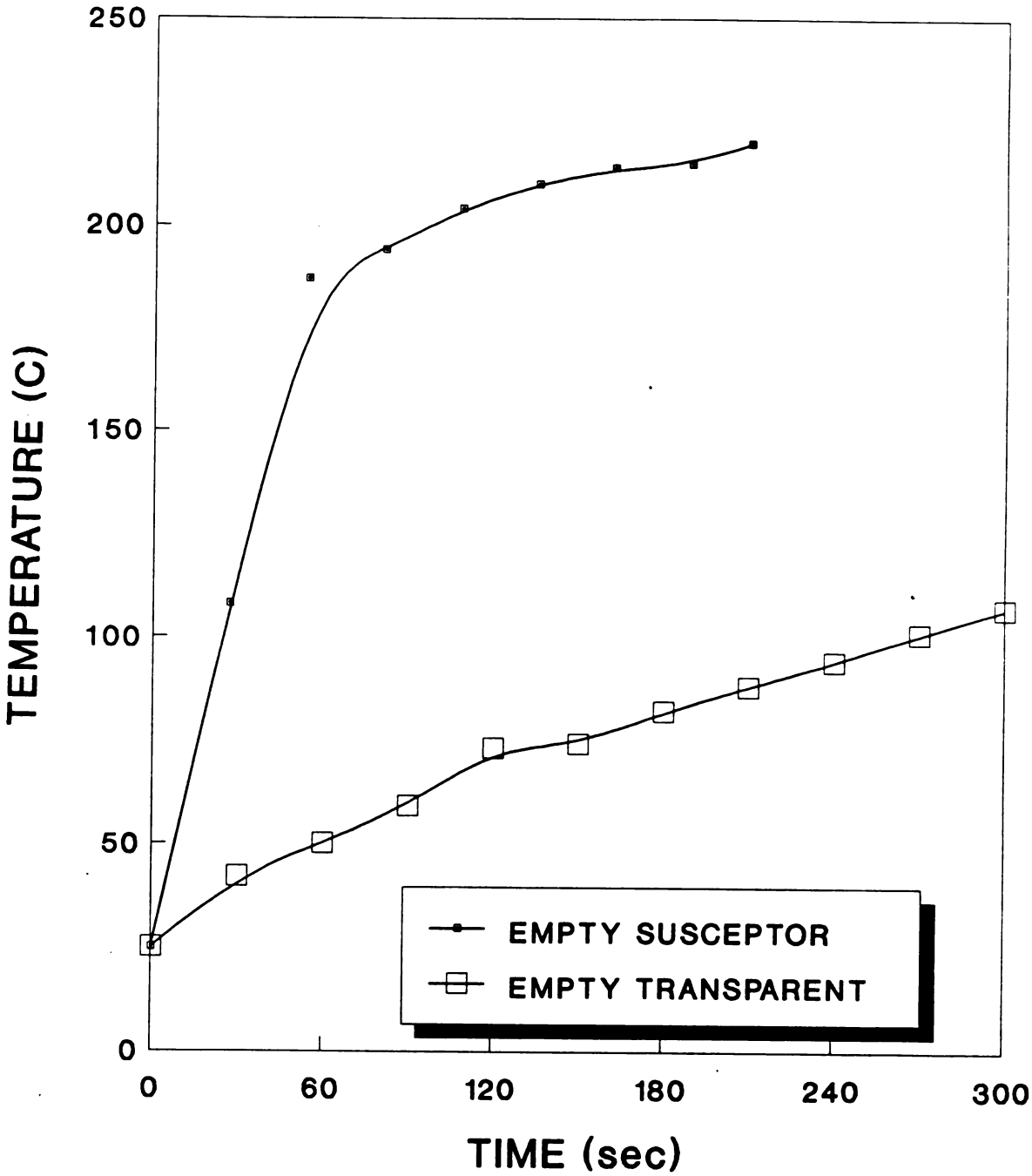


Fig. 5. Temperature profile of empty susceptor and transparent materials heated in a 700 watt microwave oven.

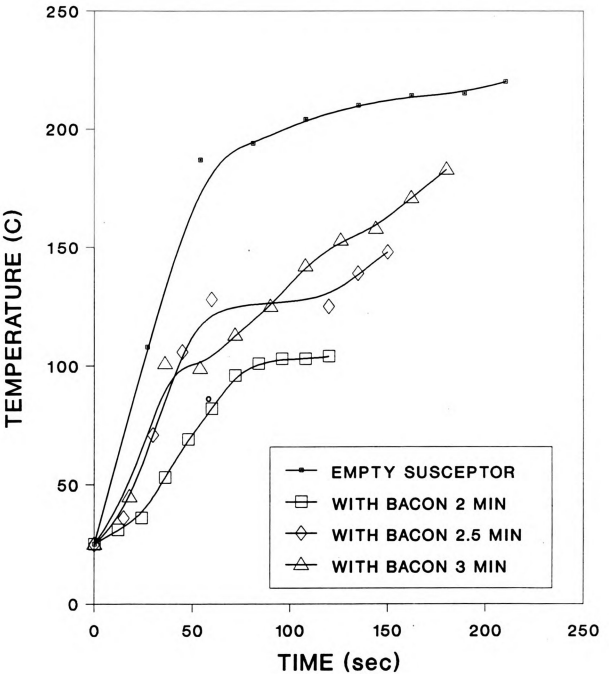


Fig. 6. Temperature profile of empty susceptor and susceptor in contact with bacon, heated in a 700 W microwave oven.

During microwaving, temperatures on the surface of the transparent packages loaded with bacon, were greater than those observed on the empty packages (Fig. 7). Higher temperatures were associated with longer cooking times. A temperature of 129°C (264°F) was obtained after microwaving for 2 min, and 159°C (319°F) during heating in the transparent packages for 4 min. A temperature of 139°C (282°F) was reached with the empty package after heating for 3 min (Fig. 7).

A susceptor acts as a secondary source of heat during microwave heating (Perry, 1986), because it absorbs microwave energy and expresses it as heat. Temperatures on the empty susceptor were found to be greater than those on the susceptor loaded with bacon, because some of the heat was consumed by bacon during cooking. Temperatures on the surface of susceptors as high as 400 - 450 °F have been observed (Denford, 1988; Labuza, 1988; Mitchell, 1989). However, transparent materials do not couple with microwaves. The dipole response of food components (e.g free water molecules, fat, ... etc) interacts with the microwave frequency, and generates heat within the food product (Mudgett, 1986). Greater temperatures were observed on the surface of transparent packages containing bacon than on the surface of the empty material, because of conduction from the bacon to the material. Overall, higher cooking temperatures were obtained using the susceptor in-package cooking method.

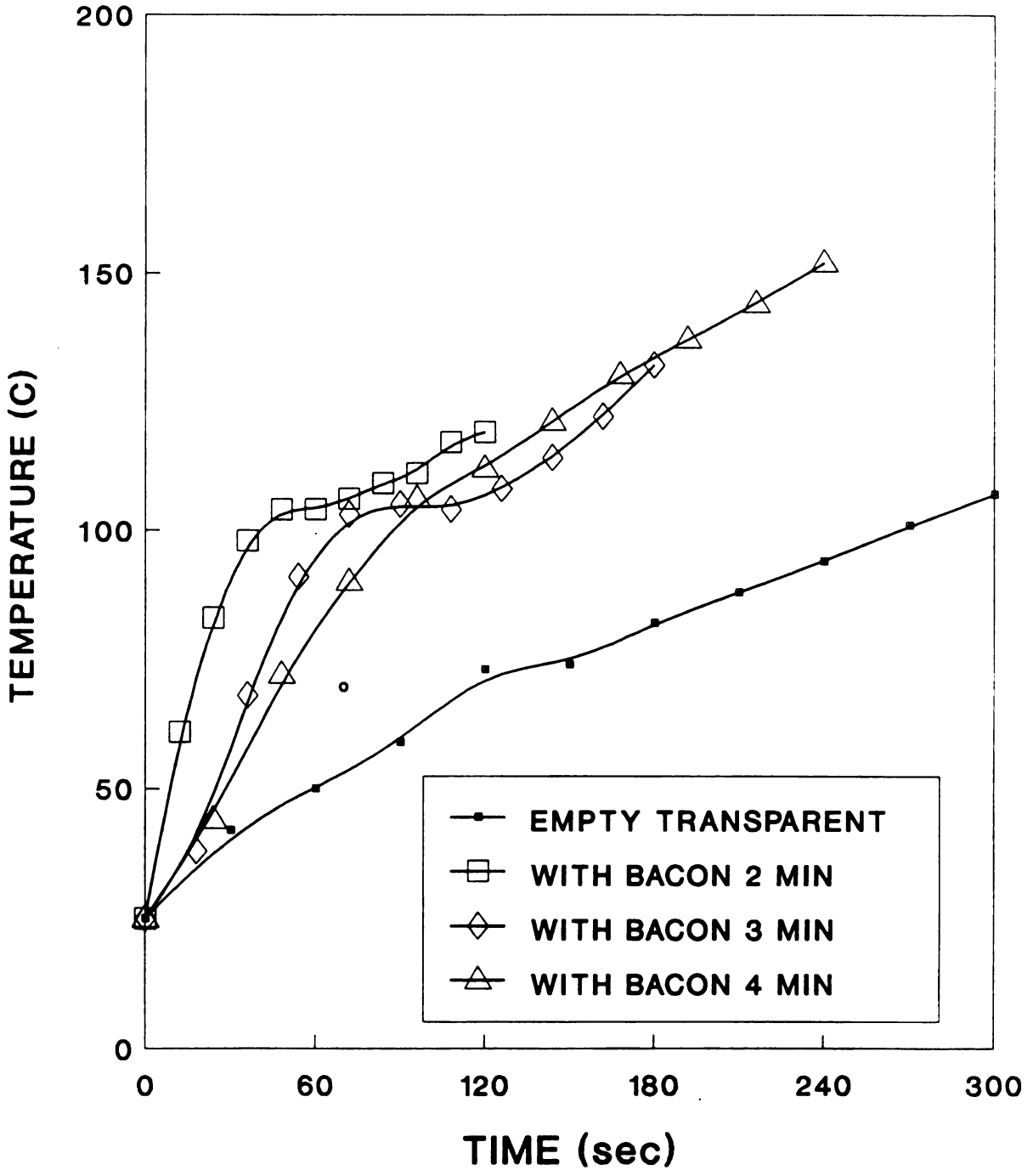


Fig. 7. Temperature profile of empty transparent package and transparent package in contact with bacon,heated in a 700 W microwave oven.

Evaluation of Degree of Doneness

An evaluation of the "degree of doneness" of the microwaved bacon was accomplished in comparison with bacon fried for 3 min. Bacon, cooked on the susceptor for 2 min had a pale, glassy appearance, and a charred look after 3 min of cooking (Fig. 8). For the bacon cooked in the transparent packages, "undercooked" and "overcooked" were obtained using cooking times of 2 and 4 min, respectively (Fig. 9).

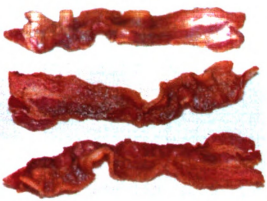
Visual evaluation of the same degree of doneness was achieved based on browning of the cooked bacon. The same degree of doneness achieved with fried bacon (3 min/side at 340°F) was obtained when samples were prepared on the susceptor for 2.5 min, and in the transparent package for 3 min (Fig. 10). Therefore, the same "degree of doneness" was obtained on the susceptor in a shorter cooking period time, in comparison to bacon prepared in the microwave transparent package and in the fry pan.

Formation of N-Nitrosodimethylamine in Cooked Bacon

The major concern associated with N-nitrosamines in cooked bacon relates to the formation of N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPYR), because these two N-nitroso compounds are regarded as health hazards, i.e. they possess carcinogenic activity (Preussmann et al., 1976; Magee et al., 1976; Sen et al., 1980). N-nitrosomorpholine (NMOR) was also formed in some samples.

Fig 8. Bacon prepared in the susceptor package using cooking times of 2, 2.5 and 3 min in a 700 W microwave oven.

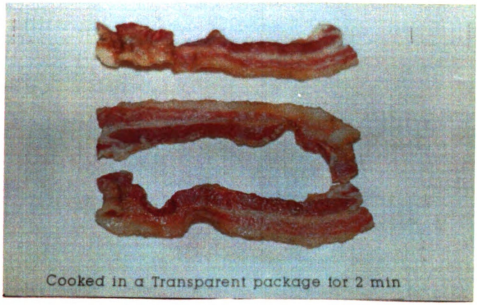
Fried at 340°F for 3 min each side



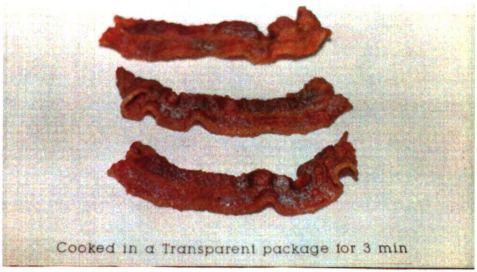
Cooked in a susceptor package for 2.5 min

Cooked in a transparent package for 3 min

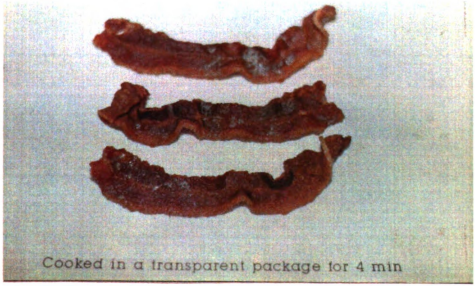
Fig 9. Bacon prepared in the transparent package using cooking times of 2, 3 and 4 min in a 700 W microwave oven.



Cooked in a Transparent package for 2 min

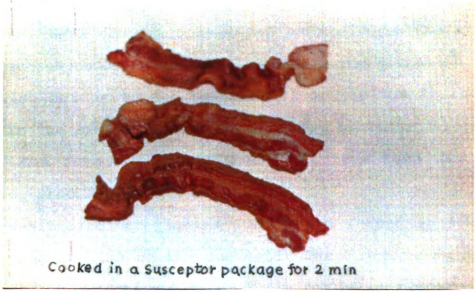


Cooked in a Transparent package for 3 min

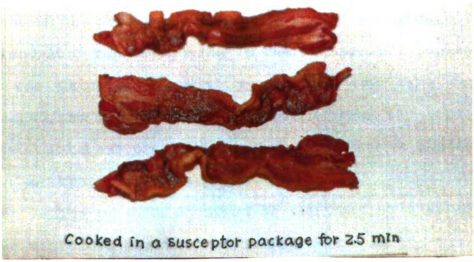


Cooked in a transparent package for 4 min

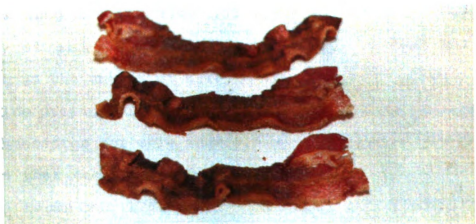
Fig. 10. Bacon prepared using three different cooking methods to the same degree of doneness.



Cooked in a susceptor package for 2 min



Cooked in a susceptor package for 2.5 min



Cooked in a susceptor package for 3 min

However, NMOR is not considered as toxic as the other N-nitroso compounds (Magee et al., 1976). The formation of NMOR may also be due to the presence of morpholine in the steam used in the smokehouse during smoking (Bussey et al., 1980). Thus, the formation of NMOR may not have been due to the cooking process.

N-Nitrosodimethylamine (NDMA) was formed in MSU bacon following heating on the susceptor for 2.5 min. No NDMA was detected at a cooking time of 2 min for bacon prepared in the transparent packages. At a cooking time of 2.5 min, NDMA was present in the greatest concentration for the susceptor-cooked samples. At 3 min of cooking in the transparent package, more NDMA was detected than at 4 min. At the same degree of doneness, the amount of NDMA in fried bacon was less than in the two microwave prepared samples (2.5 min for susceptor and 3 min in transparent package) (Table 4). Reduction in the concentration of NDMA occurred as the cooking time increased during microwave heating, which may be due to the low boiling point of NDMA (153°C). Thus, NDMA may have been vaporized at these long cooking time and elevated cooking temperatures (Gray et al., 1978). The concentration of NDMA was not as great as the amount of NPYR in the same bacon (Table 4), for samples prepared in transparent packages for 3 min, and/or in the susceptor for more than 2.5 min. Pensabene et al. (1974) found that the optimum temperature for formation of NPYR in fried bacon was 176.7°C (350°F), which might result in the

Table 4. N-Nitrosamine concentrations in cooked bacon processed in the MSU Meat Lab prepared and cooked by three different methods (residual nitrite was 21.4 ± 4.6 mg/kg).

COOKING METHOD	WT LOSS (%)	RECOVERY (%)	NDMA (μg/kg)	NPYR (μg/kg)	NMOR (μg/kg)
FRYING					
3 min/side	67	110 ± 21	0.7 ± 0.1	4.6 ± 0.4	3.1 ± 1.8
TRANSPARENT					
2 min	58	91 ± 5	ND	ND	6.6 ± 0.4
3 min	68	117 ± 21	1.5 ± 0.2	ND	4.3 ± 0.2
4 min	73	104 ± 5	0.7 ± 0.0	3.0 ± 0.1	1.4 ± 0.2
SUSCEPTOR					
2 min	51	97 ± 7	ND	ND	4.3 ± 0.4
2.5 min	67	92 ± 1	1.4 ± 0.1	2.3 ± 0.1	2.7 ± 0.1
3 min	71	95 ± 13	1.1 ± 0.0	5.6 ± 0.5	0.9 ± 0.2

ND means not detected.

vaporization of NDMA.

In the commercial bacon, NDMA was detected in all samples regardless of the cooking method. Less NDMA was found in susceptor-cooked samples in comparison to transparent cooked bacon. For bacon prepared in the transparent package, a cooking time of 4 min was found to produce the greatest concentration of NDMA for any of the cooked bacon samples, regardless of the cooking method (Table 5). The NDMA concentration in fried bacon was less than in bacon prepared in the transparent package for 3 min. However, the amount of NDMA in fried bacon was greater than for bacon cooked in the susceptor package for 2.5 min (Table 5).

Formation of N-Nitrosopyrrolidine in Cooked Bacon

For bacon processed in the MSU Meat Laboratory, no NPYR was detected after 3 min cooking in the transparent package. NPYR was detected in microwave cooked bacon which was prepared in transparent and/or susceptor materials after 4 min and 2.5 min, respectively (Table 4). More NPYR was found in susceptor-cooked bacon as cooking time increased. The concentration of NPYR in bacon prepared in the transparent material was less than pan fried bacon. Bacon cooked on the susceptor for 3 min was found to contain more NPYR than fried bacon (Table 4).

In the commercial bacon, NPYR concentrations were found to be greater in the fried samples in comparison to those prepared in the a microwave oven at all cooking times. No NPYR

Table 5. N-Nitrosamine concentrations in bacon purchased commercially and prepared by three different cooking methods (residual nitrite was 37.2 ± 16.4 mg/kg).

COOKING METHOD	WT LOSS (%)	RECOVERY (%)	NDMA (μg/kg)	NPYR (μg/kg)	NMOR (μg/kg)
FRYING					
3 min/side	64	104 ± 11	0.5 ± 0.2	6.8 ± 1.3	0.6 ± 0.2
TRANSPARENT					
2 min	50	95 ± 5	0.2 ± 0.1	ND	1.9 ± 0.4
3 min	69	101 ± 2	1.5 ± 0.2	ND	3.1 ± 0.2
4 min	74	93 ± 11	2.1 ± 0.2	1.3 ± 1.0	3.5 ± 1.2
SUSCEPTOR					
2 min	46	98 ± 7	0.5 ± 0.1	ND	1.7 ± 0.8
2.5 min	61	101 ± 4	0.3 ± 0.0	1.9 ± 0.1	4.8 ± 0.8
3 min	69	96 ± 7	0.6 ± 0.0	4.0 ± 0.8	3.7 ± 0.4

ND means not detected.

was detected in bacon prepared on the susceptor after 2 min, and in the transparent package for up to 3 min (Table 5). The greatest NPYR formation in all susceptor cooked bacon was observed at a cooking time of 3 min. However, more NPYR was found in susceptor-cooked bacon at 2.5 min than in bacon cooked in the transparent package for 4 min (Table 5). No NPYR was detected in either the microwaved bacon prepared in the transparent package up to 3 min of cooking, or on susceptors for 2 min (Table 4, 5).

A comparison of the NPYR concentrations in cooked bacon, prepared by the three different cooking methods to the same degree of doneness, was developed. Fried bacon contained more NPYR than the bacon prepared in either microwave method for both the MSU (Fig. 11) and commercial (Fig. 12) bacon samples. More NPYR was formed using the susceptor cooking method than in the transparent cooked samples (Fig. 11 and 12). The greater concentration of NPYR in the susceptor cooked samples was probably due to the higher temperature during cooking (Fig. 13). NPYR may be formed in bacon when free proline is nitrated to N-nitrosoproline, and in turn thermal decarboxylated to yield NPYR (Bharucha et al., 1979; Lee et al., 1983b). The concentration of NPYR in fried bacon was greater than in the microwave cooked samples, which is probably due to the time and temperature dependent decarboxylation. When the temperature reaches the necessary limit for formation of N-nitrosamines, the concentration of

Fig. 11. Concentrations of N-nitrosamines in bacon processed in the MSU Meat Lab. and prepared using three different cooking methods to the same degree of doneness.

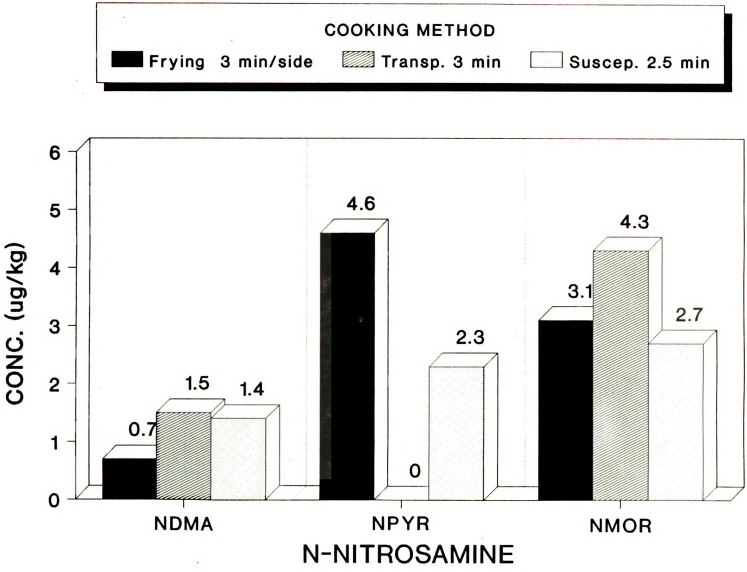
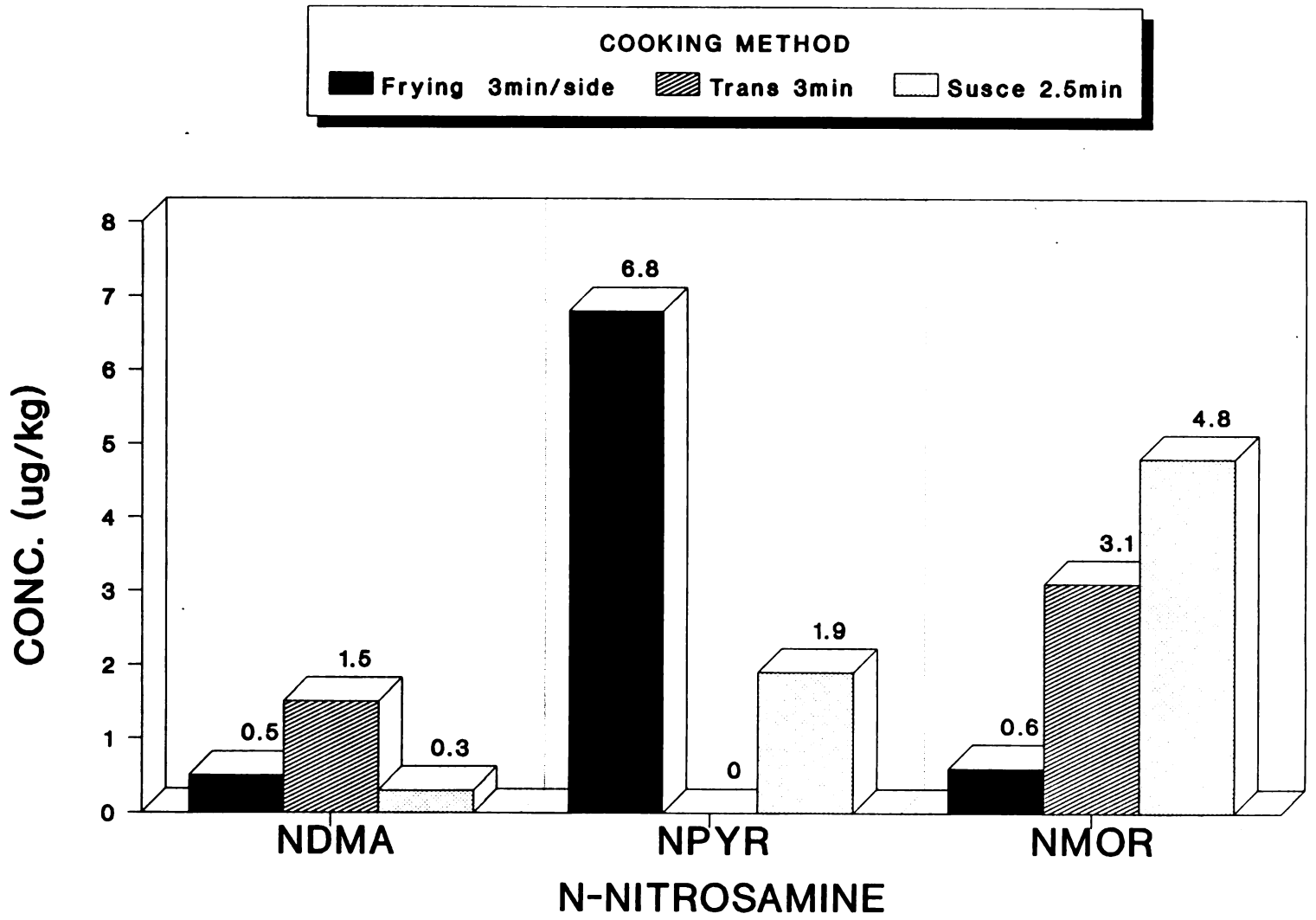

COOKING METHOD
Frying 3 min/side
Transp. 3 min
Suscep. 2.5 min
CONC. (ug/kg)
0
1
2
3
4
5
6
0.7
1.5
1.4
4.6
0
2.3
3.1
4.3
2.7
NDMA
NPYR
NMOR
N-NITROSAMINE

Fig. 12. Concentrations of N-nitrosamines in bacon purchased commercially and prepared using three different cooking methods to the same degree of doneness.


COOKING METHOD
Frying 3min/side
Trans 3min
Susce 2.5min
CONC. (ug/kg)
0
1
2
3
4
5
6
7
8
0.5
1.5
0.3
6.8
0
1.9
0.6
3.1
4.8
NDMA
NPYR
NMOR
N-NITROSAMINE

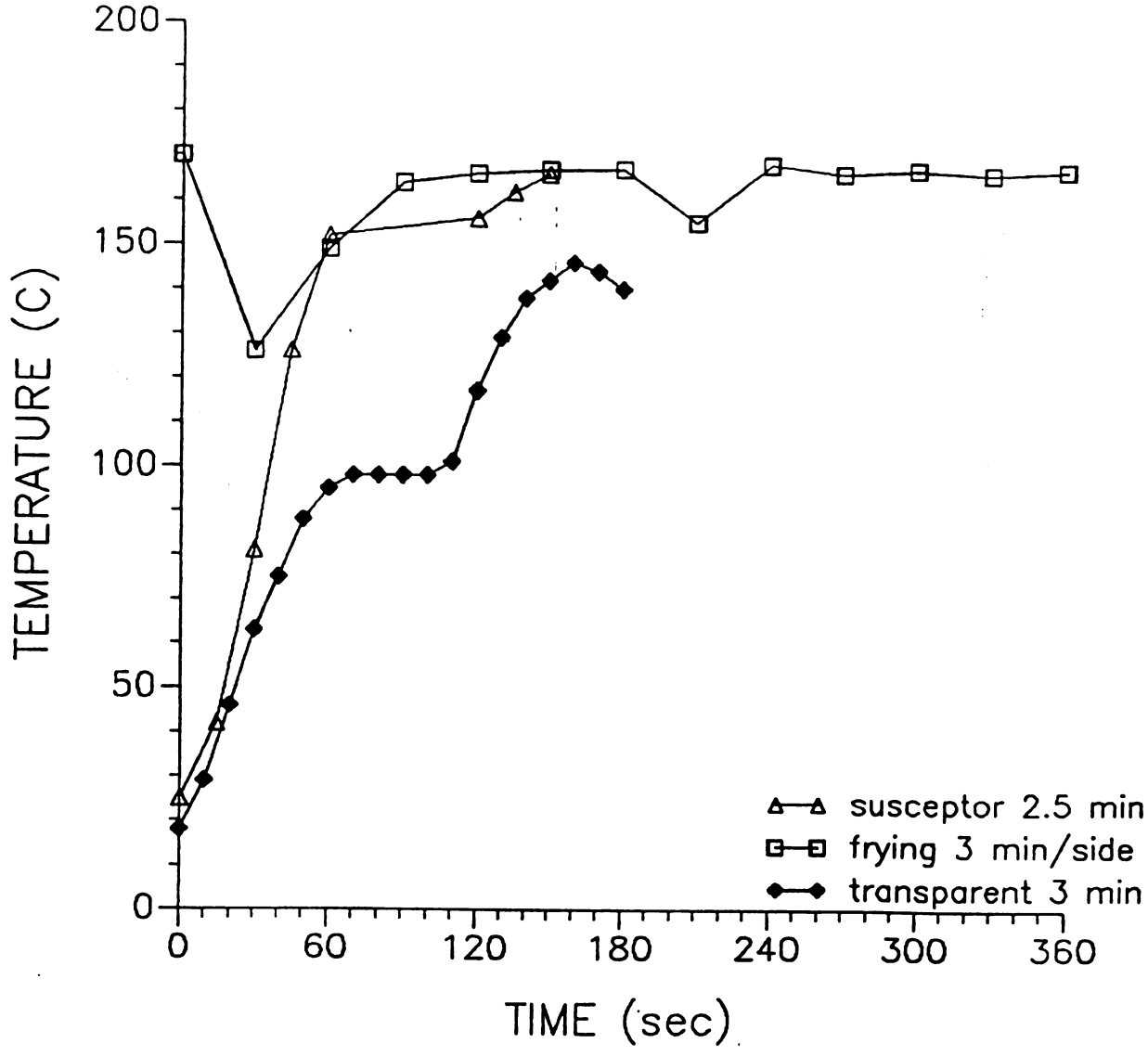


Fig. 13. Temperature profile of bacon prepared using three different cooking methods to the same degree of doneness.

NPYR increases with heating duration (Pensabene et al., 1974; Lee et al., 1983b). Therefore, the 6 min total cooking duration used in frying, compared to cooking times of 2.5 min for the susceptor and 3 min in the transparent package (Fig. 13), resulted in a greater NPYR concentration in the fried bacon.

Effect of Weight Loss on Formation of N-Nitrosamines

The majority of N-nitrosamines are formed after the bulk of water has vaporized (Pensabene et al., 1974; Coleman, 1978). The relationship between weight loss and formation of NPYR and NDMA was determined. Formation of NDMA increased as weight loss increased during microwaving, R^2 values of 0.923 and 0.909 were obtained for the susceptor and transparent cooked bacon, respectively (Fig. 14). More NDMA was found in susceptor cooked bacon relative to the amount of weight loss up to 65%. More NDMA was developed as the weight loss increased in microwave transparent in-package cooking than in susceptor cooking (Fig. 14). The lower quantity of NDMA in susceptor cooked bacon might be due to the evaporation of NDMA at high temperatures such as achieved on the surface of the susceptor. During cooking, the bulk of the water is vaporized which results in weight loss. NDMA may also evaporate, which results in less NDMA in the cooked bacon.

More NPYR formation was also found to be correlated with the increase of weight loss. The correlation coefficient

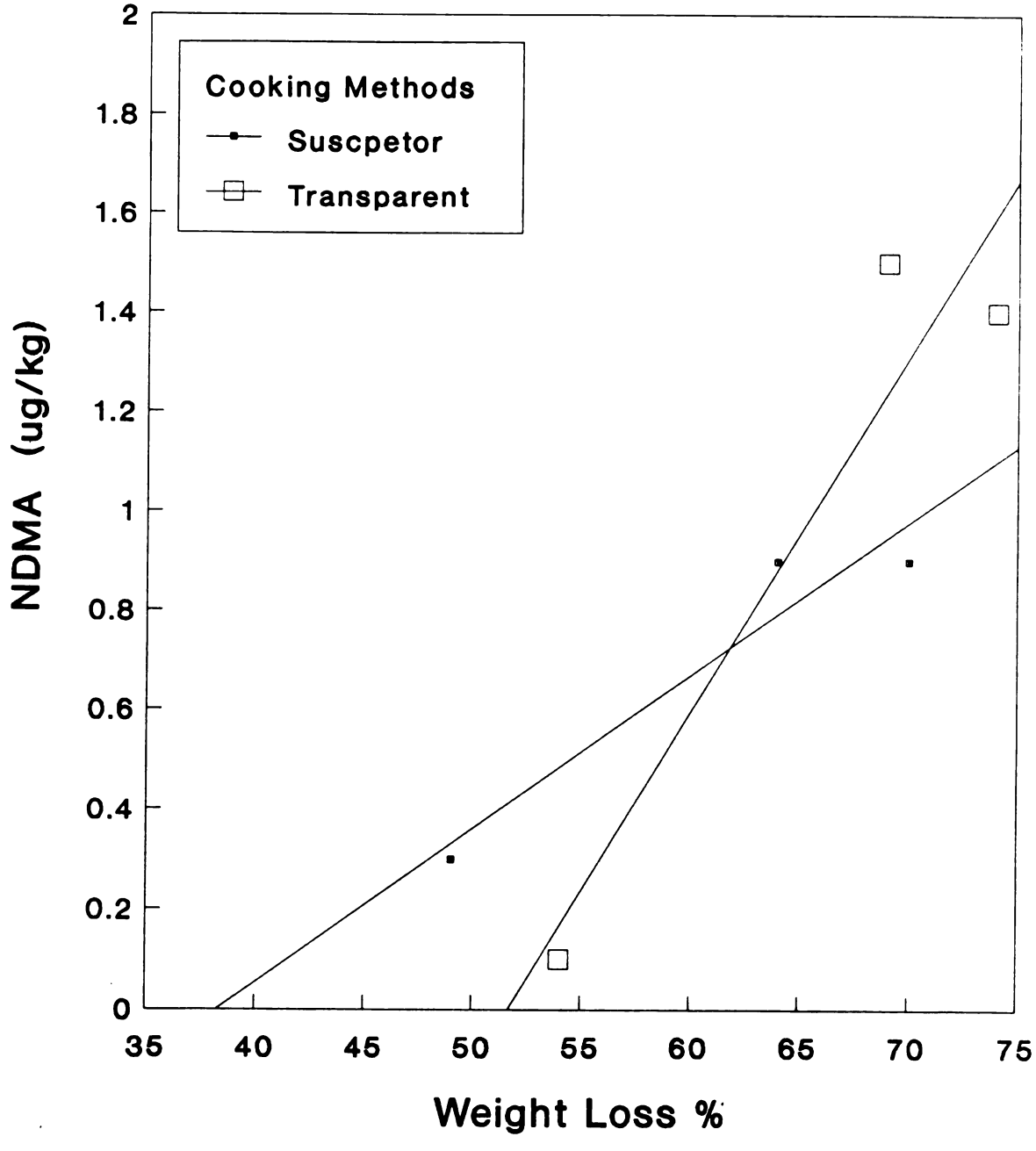


Fig. 14. Relationship between formation of NDMA and weight loss using microwave in-package cooking methods (fried bacon had an average of 0.6 μg/kg NDMA formed with an average of 66% weight loss)

between NPYR formation and weight loss using the different cooking methods (susceptor and transparent cooking) were 0.904 and 0.754, respectively (Fig. 15). A greater amount of NPYR was found in susceptor cooked bacon than is samples prepared in the microwave transparent packages with the same weight loss. In addition, the formation of NPYR was influenced more by weight loss during susceptor cooking compared to transparent in-package cooking, which means that more NPYR was formed in susceptor cooked bacon even though the weight loss was the same (Fig. 15).

Correlation of N-Nitrosamine Formation and Temperature-Time History

Since formation of N-nitrosamines is highly related to cooking temperature and time, the Temperature-Time History is an important parameter which can be used to determine the relationship between formation of N-nitrosamines and cooking methods. The effect of cooking methods on formation of NPYR was determined by developing a correlation between the concentration of NPYR detected versus the Temp-Time History of the cooking methods. The R^2 values were observed to be 0.936 in susceptor cooking, 0.736 in frying and 0.783 in microwave transparent cooking. The susceptor cooking method created more NPYR compared to frying and microwave transparent cooking based on the same Temp-Time History. Frying was found to be similar to the transparent cooking (Fig. 16). More NPYR was

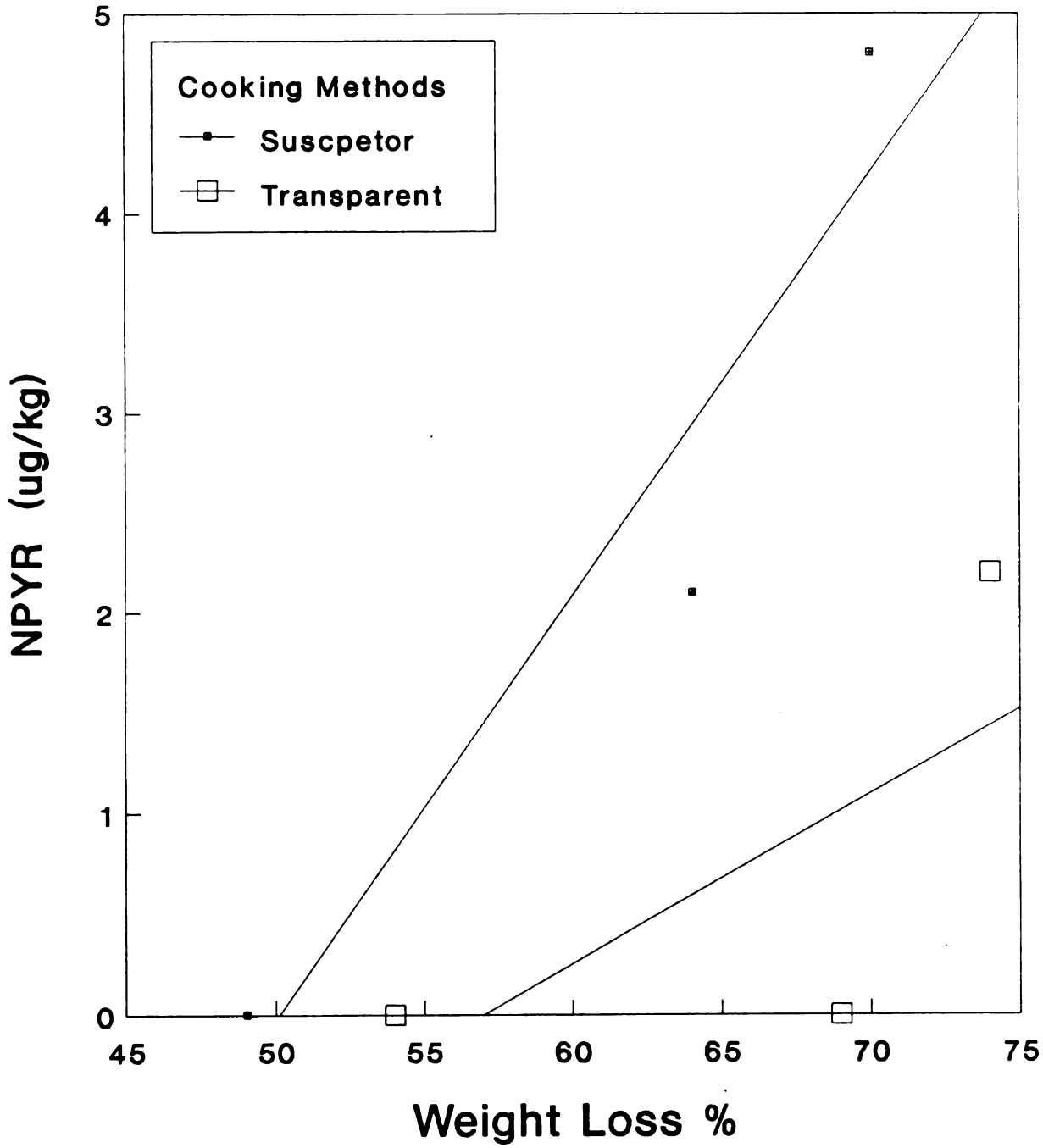


Fig. 15. Relationship between formation of NPYR and weight loss using microwave in-package cooking methods (fried bacon had an average of 5.7 μg/kg NPYR formed with an average of 66% weight loss)

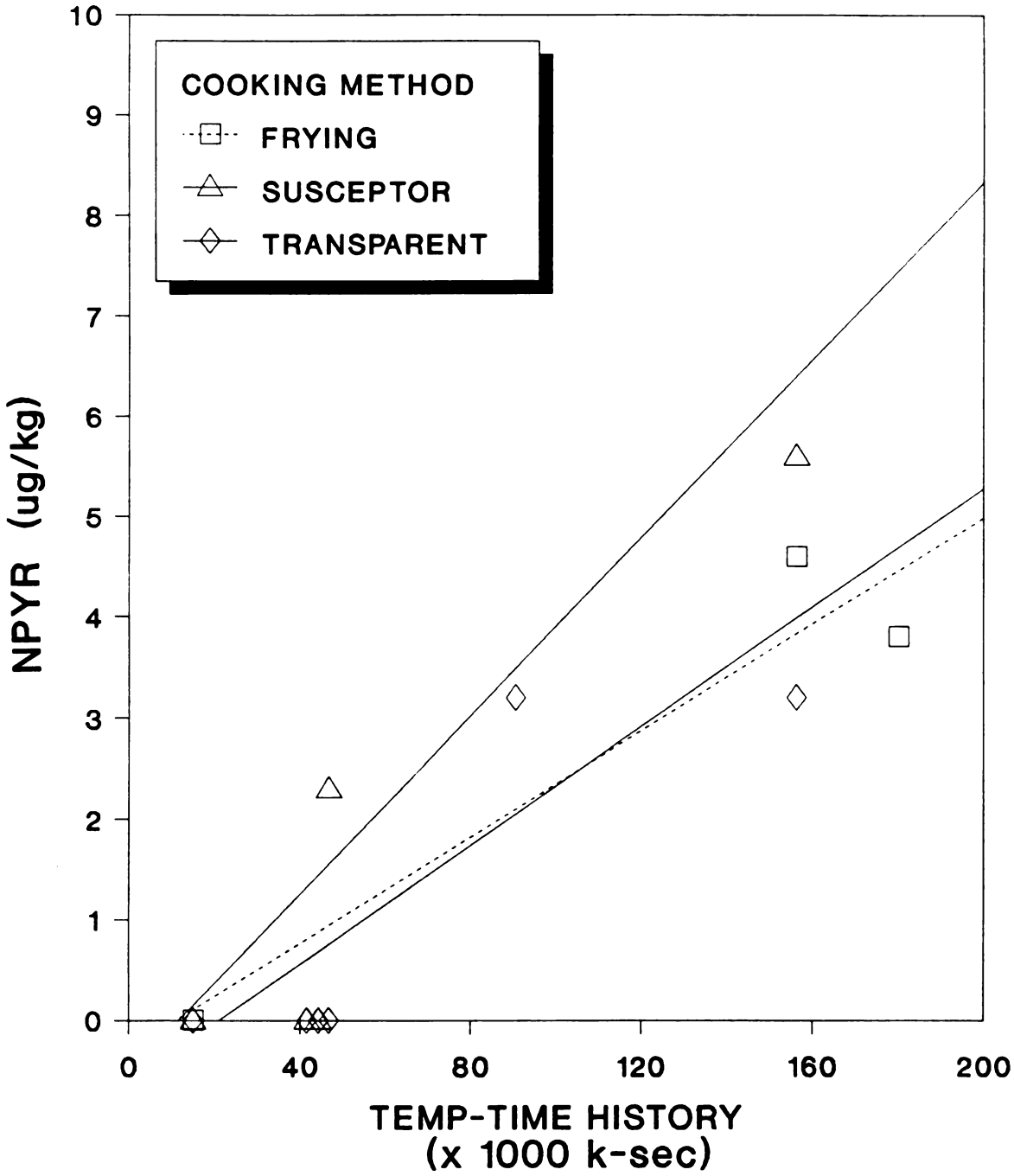


Fig. 16. Correlation of the formation of NPYR in bacon and the Temperature-Time History of three different cooking methods to the same degree of doneness.

formed in the susceptor in-package cooking method, which may have occurred because the product was heated by interaction with the microwave energy, as well as conduction from the hot surface of the susceptor. However, with Temp-Time History values greater than 150,000 K^{o}-sec, transparent in-package resulted in more NPYR formation than frying at the same Temp-Time History (Fig. 16), because better heat penetration is expected during microwaving. Since, formation of N-nitrosamines is primarily affected by the time and temperature of cooking (Pensabene et al., 1974; Wasserman et al., 1978; Bharucha et al., 1979; Theiler et al., 1981), a thermal process which elevates the product temperature allows more N-nitrosamines to be formed. Microwave energy heats products from the outside, which is similar to conventional heating (Perry, 1986). The difference in the heating patterns is that the heated air of a conventional oven gradually increases the temperature on foods, however, microwave energy due to its penetration ability heats foods more quickly (Martin, 1988). Moreover, bacon prepared on the susceptor, was cooked due to product interaction with the microwave energy, and susceptor induced heating (Perry, 1986). Therefore, susceptor in-package cooking would be expected to produce more N-nitrosamines.

Effect of Residual Nitrite on Formation of N-Nitrosamine

The formation of N-nitrosamines are not only influenced by the cooking temperature and duration (Pensabene et al.,

1974; Wasserman et al., 1978; Bharucha et al., 1979; Theiler et al., 1981), but also by the residual nitrite concentration in the raw bacon. At the same degree of doneness, fried bacon had the greatest concentration of NPYR and NDMA, compared to both microwave cooked products at two levels of ingoing nitrite (120 and 200 mg/kg). (Table 6). More NPYR was formed in cooked bacon with 200 mg/kg ingoing sodium nitrite than sample with 120 mg/kg. Susceptor-cooked bacon had more NPYR than transparent-cooked samples (Table 6).

NDMA was detected in lesser concentrations than NPYR in the same cooked bacon, and a greater amount of NDMA was found associated with the increased nitrite in the curing process (Table 6). For NDMA, an average increase of 41% (average from 0.73 to 1.03 μg/kg) was obtained when the ingoing nitrite concentration was 120 to 200 mg/kg, respectively. Susceptor-cooked bacon contained more NDMA than bacon prepared in the transparent package at both nitrite levels, though, lesser amounts than in the fried samples (Table 6).

An increase of 51% in the concentration of NPYR (from 4.6 to 7.4 μg/kg) was observed in fried bacon, at the higher nitrite level (Fig. 17). However, no significant difference in NPYR formation was observed for microwave-cooked samples at the different ingoing nitrite levels (Fig. 17).

Statistical Analysis

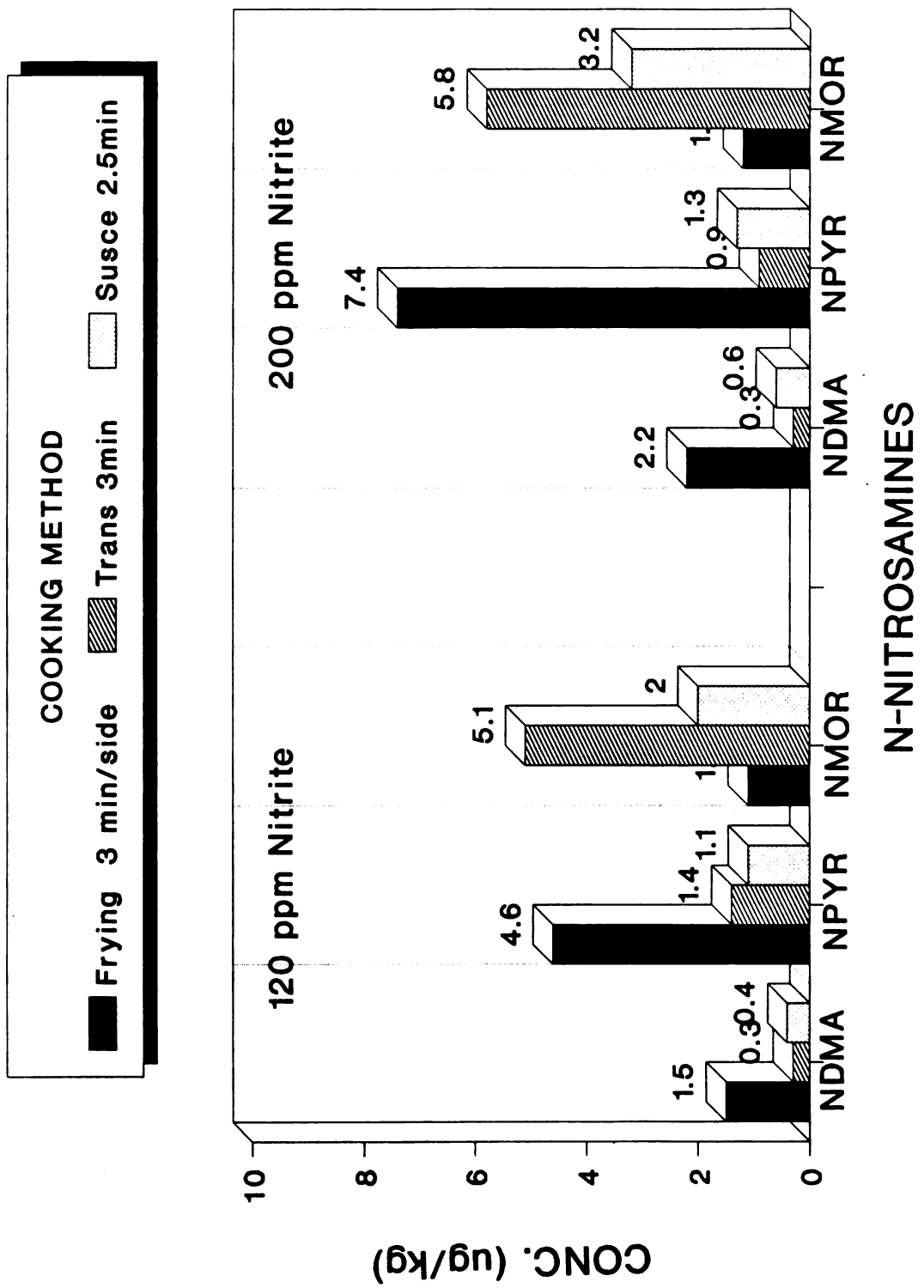
A paired comparison test (Gill, 1978) was used to compare

Table 6. N-Nitrosamine concentrations in cooked bacon (MSU Meat Lab.) processed with two different ingoing nitrite levels and prepared using three different cooking methods.

RESIDUAL NITRITE (mg/kg)	COOKING METHOD	WT LOSS (%)	RECOVERY (%)	NDMA (μg/kg)	NPYR (μg/kg)	NMOR (μg/kg)
	FRYING 3 min/ side	68	103± 6	1.5±0.1	4.6±0.1	1.1±0.7
7.8±2.7[a]	SUSCEPTOR 2.5 min	71	96±11	0.4±0.1	1.1±0.1	2.0±0.3
	TRANSPARENT 3 min	73	101± 7	0.3±0.0	1.4±0.4	5.1±0.1
	FRYING 3 min/ side	68	109± 8	2.2±0.0	7.4±0.7	1.2±0.5
63.1±1.9[b]	SUSCEPTOR 2.5 min	55	104± 4	0.6±0.1	1.3±0.6	3.2±0.4
	TRANSPARENT 3 min	63	104± 1	0.3±0.0	0.9±0.1	5.8±0.2

a. 120 mg/kg of ingoing nitrite concentration
b. 200 mg/kg of ingoing nitrite concentration

Fig. 17. Concentration of N-nitrosamines in bacon processed using two nitrite concentrations and prepared using three different cooking methods to the same degree of doneness.


COOKING METHOD
Frying 3 min/side
Trans 3min
Susce 2.5min
120 ppm Nitrite
200 ppm Nitrite
CONC. (ug/kg)
0
2
4
6
8
10
1.5
0.3
0.4
4.6
1.4
1.1
1
5.1
2
2.2
0.3
0.6
7.4
0.9
1.3
1
5.8
3.2
NDMA
NPYR
NMOR
NDMA
NPYR
NMOR
N-NITROSAMINES

the influence of the different cooking methods and nitrite levels on the formation of NPYR. No significant differences were observed between the different microwave methods at 120 mg/kg ingoing nitrite. However, significant differences ($\alpha < 0.01$) between fried and susceptor-cooked, and fried and transparent-cooked samples were obtained (Table 7). For the susceptor in-package cooking, the increased quantity of NPYR formed was associated with the increased nitrite levels in the curing brine (Table 6), however, no significant difference of the NPYR formation between two nitrite levels (120 and 200 mg/kg) in susceptor cooking was observed (Table 7). A 51% increase in NPYR was observed in the fried bacon as the concentration of nitrite was increased in the brine (Fig. 15), though this was not a significant difference ($\alpha < 0.1$) compared to the brine containing the lesser amount of nitrite used (Table 7).

Table 7. Statistical analysis using Boferroni t-tests to determine the effect of different ingoing nitrite concentrations in the brine and cooking methods on the formation of NPYR.

Treatment	Ingoing Nitrite (mg/kg)	Cooking Method
1	120	Frying
2	120	Microwave Susceptor
3	120	Microwave Transparent
4	200	Frying
5	200	Microwave Susceptor
6	200	Microwave Transparent

Contrasts	t_B
1. TRT 1[a] vs TRT 2	2.27
2. TRT 1 vs TRT 3	2.08
3. TRT 4 vs TRT 5	3.96***
4. TRT 4 vs TRT 6	4.22***
5. TRT 1,2,3 vs TRT 4,5,6	-0.94
6. TRT 1 vs TRT 4	-1.82
7. TRT 2 vs TRT 5	-0.13
8. TRT 3 vs TRT 6	-0.32

replication (r) = 4
no. of contrasts (m) = 8
degree of freedom (df) = 18

*	$\alpha = 0.10$	$t_{0.050} = 2.775$
**	$\alpha = 0.05$	$t_{0.025} = 3.095$
***	$\alpha = 0.01$	$t_{0.005} = 3.822$

a. TRT 1 means treatment 1

CONCLUSION

The temperature of the empty susceptor increased rapidly up to 100°C (212°F) at 30 seconds of microwaving. Temperatures in excess of 220°C (430°F) were observed on the surface of the empty susceptor after 3.5 min. Lower temperatures were observed on the surface of the susceptor when loaded with bacon, because the heat was consumed by the bacon during cooking. A different heating rate was found when the transparent package was used to prepare the bacon. Higher temperatures were obtained when the package was filled with bacon, due to the heat generated by dipolar interaction in the food. Final package/product temperatures of 166°C and 143°C (331°F and 290°F) were observed after 2.5 min microwaving on the susceptor and 3 min on the transparent material, respectively. Therefore, cooking in the susceptor packages was more efficiently.

Cooking to the same degree of doneness (as fried bacon) was achieved when samples were cooked on the susceptor material for 2.5 min and for 3 min in the transparent package in a microwave oven (700 Watt, full power). Microwave cooking methods reduced the time necessary to accomplish the same degree of doneness as frying. A shorter preparation time was associated with susceptor cooking compared to transparent in-package cooking.

No NPYR was found in bacon samples cooked on the

susceptor for 2 min and in the transparent package for up to 3 min of cooking. More NPYR was produced in fried bacon than bacon cooked to the same degree of doneness in a microwave oven. Smaller amounts of NPYR were found in bacon cooked in the transparent package compared to samples cooked in the susceptor package. Because of the higher temperatures associated with susceptor cooking, more N-nitrosamines were formed in comparison to cooking in the transparent packages. Greater quantities of NPYR were associated with longer cooking times. NDMA was found in smaller concentrations compared to NPYR in the same bacon. The higher temperatures necessary for the formation of NPYR may cause vaporization of NDMA, and resulted in smaller observed concentrations (Pensabene et al., 1974).

A temperature-time history was used to estimate the effect of cooking methods on NPYR formation. Microwave susceptor cooking was found to more efficiently produce NPYR, because the microwave energy penetrates deeper into the food (Martin, 1988) and the susceptor acts as a second heat source (Perry, 1986). The formation of NPYR in bacon cooked by frying and transparent in-package microwave cooking was similar, when the values of temp-time history was less than 150,000 (K^{o}-sec). However, at a greater temp-time history, transparent in-package cooking was found to allow more NPYR formation than frying. Microwave cooking methods more efficiently produced NPYR, possibly because of the better heat penetration compared

to frying.

The effect of nitrite concentration on the formation of NPYR was determined using 120 and 200 mg/kg of ingoing nitrite concentrations. A 51 % increase in NPYR was found in pan fried bacon. However, no significant increase in NPYR formation was observed in microwave cooked bacon, regardless of the package type. A significant difference in NPYR concentration was found between frying and both microwave in-package cooking methods ($\alpha < 0.01$) at 200 mg/kg nitrite treatment. However, increased nitrite concentration in the curing brine, did not significantly influence NPYR formation in bacon prepared using the same cooking method.

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