Influence of Heme Pigments, Nitrite, and Non-Heme Iron on Development of Warmed-over Flavor (WOF) in Cooked Meat

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Removal of meat pigments and addition of 156 mg/kg of nitrite significantly ($P < 0.001$) inhibited lipid oxidation in cooked meat, which suggested that heme pigments may catalyze autoxidation. Taste panel evaluation confirmed the beneficial effects of removal of heme pigments and addition of nitrite as a means of controlling the development of WOF. The percentage of bound heme iron in fresh meat pigment extract was slightly over 90% while the level of free non-heme iron was less than 10%. Cooking, however, released a significant amount of non-heme iron from bound heme pigments, which accelerated lipid oxidation in cooked meat. Thus, the increased rate of lipid oxidation in cooked meat is due to the release of non-heme iron during cooking, which catalyzes lipid oxidation. Addition of 2% ethylenediaminetetraacetic acid was shown to effectively chelate the non-heme iron and, thus, significantly reduced lipid oxidation.

It has been generally accepted that hemoproteins and other iron porphyrins accelerate lipid oxidation, with the hemoproteins being implicated as the major prooxidants of lipid oxidation in meat products (Tappel, 1952; Watts, 1954; Younathan and Watts, 1958; Maier and Tappel, 1959). In recent years, however, Sato and Hegarty (1971) and Love and Pearson (1974) have presented data suggesting that non-heme iron is the major prooxidant in cooked meat. They concluded that meat pigments per se have no catalytic effect on lipid oxidation in cooked meats. Never-the-less, a direct role for myoglobin upon lipid oxidation in meat is still widely accepted (Greene, 1969; Govindarajan et al., 1977).

Even though non-heme iron has been shown to be a prooxidant in cooked meat, its origin is unknown. Thus, the primary objective of this investigation was to determine the role, function, and source of non-heme iron as a prooxidant of lipid oxidation in cooked meat. In addition, the role and function of meat pigments and nitrite in the development of warmed-over flavor (WOF) in cooked meat were also investigated.

MATERIALS AND METHODS

Source of Meat. The beef and chicken used in this study were obtained from Michigan State University Meat and Poultry Processing Laboratories. Portions of longissimus dorsi (L-D) muscle were excised from beef carcasses at 24 h postmortem. Thigh (dark meat) and breast (white meat) meat were removed from the chicken carcasses at 24 h postmortem.

Sample Preparation. The study was divided into two stages. The first part of this study was conceived to investigate the effect of removal of meat pigments and/or the addition of nitrite to control the development of oxidized flavor in cooked meat. Thus, the meat (beef, chicken dark and white meat) was cut into pieces, ground first through a $^3/_8$ in. and later through a $^3/_{16}$ in. plate. Each kind of meat was divided into two groups. Muscle pigments from one group were removed by extraction with deionized water at 4 °C for 24 h with several volumes of water. Then the extracted meat fibers were placed on cheese cloth and repeatedly extracted with water until they were virtually devoid of pigments as judged by the absence of color in the remaining tissue and the lack of turbidity

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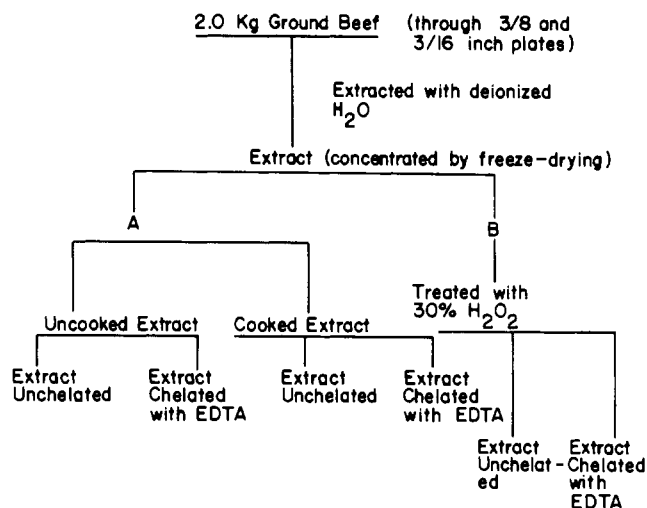
Table I. Design and Formulation of Treatments[a]

meat type	code no.	treatments and formulations
beef	A	beef with pigment, no nitrite
	B	beef with pigment, plus nitrite
	C	beef without pigment, no nitrite
	D	beef without pigment plus nitrite
chicken, dark meat	A	dark meat with pigment, no nitrite
	B	dark meat with pigment plus nitrite
	C	dark meat without pigment, no nitrite
	D	dark meat without pigment plus nitrite
chicken, white meat	A, B, C, D	same treatments were used as for dark meat

[a] Nitrite was added at a level of 156 ppm. Two hundred grams of meat was used for each treatment. Samples were cooked and stored at 4 °C for 48 h. There were four replicates for each treatment.

in the water extract. The unextracted samples were used as controls. Four experimental treatments were designed for each type of meat as shown in Table I. Each experimental treatment consisted of 200 g of meat that was mixed with 100 mL of distilled deionized water. In the nitrite-treated samples, the level of nitrite was 156 mg/kg.

The second part of the experiment was conducted using beef pigment extract to assess the relative contribution of heme and non-heme iron on development of WOF in cooked meat. The extracted meat pigments were concentrated in a Stokes freeze-drier. Then the concentrated extract was divided into lots A and B as shown in Figure 1. The extract from lot A was further subdivided into two equal parts. One part was cooked and the other part was used as an uncooked control. Half of both the cooked and uncooked extract was treated with 2% EDTA in order to chelate the non-heme iron. Extract B was treated with 30% H_2O_2 to destroy the pigment and to release the non-heme iron (Wills, 1966; Kwoh, 1970). One part of the H_2O_2-treated extract was again chelated with 2% EDTA. The unchelated extracts served as controls. Each extract was added back to a constant weight of meat residue and cooked in retortable pouches. The design of the experimental treatments is shown in Table II.

Cooking. The treated samples were packed in retortable pouches, cooked in boiling water to an internal temperature of 70 °C, and stored at 4 °C for 48 h. TBA numbers and/or taste panel evaluation (stage 1) of the

2.0 Kg Ground Beef (through 3/8 and 3/16 inch plates)

Extracted with deionized H₂O

Extract (concentrated by freeze-drying)

A

B

Uncooked Extract   Cooked Extract

Treated with 30% H₂O₂

Extract Unchelated   Extract Chelated with EDTA   Extract Unchelated   Extract Chelated with EDTA

Extract Unchelated   Extract Chelated with EDTA

Figure 1. Preparation and design of experiment to compare the effect of heme and non-heme iron on the development of WOF.

Table II. Experimental Treatments to Compare the Effect of Heme and Non-heme Iron on the Development of WOF

treatment no.	preparation of exptl treatments
1	residue + total meat pigment
2	residue + total meat pigment (chelated)[a]
3	residue + total cooked meat pigment
4	residue + total cooked meat pigment (chelated)[a]
5	residue + H₂O₂ treated meat pigment
6	residue + H₂O₂ treated meat pigment (chelated)[a]
7	residue + deionized water

[a] Non-heme iron was chelated using 2% EDTA.

cooked samples were used to determine the extent of lipid oxidation as influenced by either meat pigment, nitrite or non-heme iron.

TBA Test. The distillation method of Tarladgis et al. (1960) was utilized to measure the development of oxidative rancidity by the TBA test. However, a modification in the distillation procedure as described by Zipser and Watts (1962) was used for the nitrite-treated samples, since nitrite interferes with the distillation step by nitrosation of malonaldehyde (Hougham and Watts, 1958; Younanthan and Watts, 1959). In the modified method of Zipser and Watts (1962), sulfanilamide is added to the samples containing nitrite. TBA numbers were expressed as milligrams of malonaldehyde/kilogram of meat.

Tastes Panel Evaluation. Sensory evaluation was carried out after 48 h storage of the cooked meat at 4 °C by four trained panelists. At each setting, all panelists were presented with four different coded samples representing different treatments. A control sample consisting of freshly cooked meat was also presented along with the treated samples. All experimental samples were reheated in boiling water to an approximate internal temperature of 70 °C and served while hot. The panel scoring system was as follows: 1 = very pronounced WOF; 2 = pronounced WOF; 3 = moderate WOF; 4 = slight WOF; and 5 = no WOF.

Lipid Analyses. Total lipids were extracted by the procedure of Folch et al. (1957). Separation of the neutral lipids and phospholipids was achieved by the method described by Choudhury et al. (1960). The percentage of total lipids, neutral lipids, and phospholipids in the raw meat samples were then calculated.

Preparation of Methyl Esters. Triglycerides and phospholipids used for fatty acid analysis were converted to methyl esters by the boron trifluoride/methanol procedure as described by Morrison and Smith (1964). GLC analysis of fatty acid methyl esters was performed using a Perkin-Elmer Model 900 gas chromatograph as described by Igene and Pearson (1979).

Determination of Heme and Non-heme Iron. The concentration of heme and non-heme iron in the extracted meat pigments was determined using an atomic absorption spectrophotometer (Instrumentation Laboratory, Inc., Lexington, MA). The samples were prepared by nitric and perchloric acid digestion before analyzing for iron. Non-heme iron was separated from heme iron first by chelating with 2% EDTA and then by precipitating the bound heme iron using 12.5% of trichloroacetic acid (TCA). After centrifugation, the supernatant was removed for the determination of free non-heme iron. The difference between total iron and free non-heme iron represented the amount of heme iron in the extract. The free non-heme iron as determined also included free heme iron (any heme iron from which the protein has been cleaved off), but the amount would be minor in the unheated pigment extract.

Statistical Methods. TBA values and taste panel scores were subjected to analysis of variance using a Control Data Corporation (CDC) 6500 computer. Correlation coefficients were also calculated, and their significance was determined by the "r" distribution table from Snedecor and Cochran (1973).

RESULTS AND DISCUSSION

Lipid Composition of Meat Samples. The levels of lipids and moisture in both the extracted and nonextracted meat samples were determined and are presented in Table III. This was done to enable a meaningful assessment of the effect of pigments on development of WOF. Total lipids and triglycerides tended to be slightly lower in the pigment-extracted meat than in that containing pigment, except for chicken white meat. The amounts of phospholipids were 0.58 and 0.60% in beef with and without pigments, respectively. The corresponding levels of phospholipids in chicken dark meat were 0.86 and 0.80%, respectively. The concentration of phospholipids was considerably lower in chicken white meat than in chicken dark meat, with values of 0.49 and 0.54% in the chicken white meat with and without pigments, respectively. There were only minor differences in the levels of moisture between samples with and without pigments. Thus, the

Table III. Levels of Lipids and Moisture in Meat Samples (Percent Fresh Tissue)[a]

	beef		dark meat		white meat	
component	with pigment	without pigment	with pigment	without pigment	with pigment	without pigment
total lipids	4.39	3.23	4.80	3.77	1.63	1.87
triglyceride	3.76	3.61	3.90	2.97	1.14	1.30
phospholipid	0.58	0.60	0.86	0.80	0.49	0.54
moisture	76.01	78.57	74.13	78.23	80.22	80.51

[a] Results represent duplicate determinations.

Table IV. Summary of Fatty Acid Composition of the Triglycerides in Beef, Chicken Dark, and White Meat[a]

fatty acid	beef with pigment	beef without pigment	dark meat with pigment	dark meat without pigment	white meat with pigment	white meat without pigment
% saturated	47.64	43.96	25.99	28.59	31.41	29.39
% monoenoic	48.33	50.69	49.70	48.78	45.00	49.08
% dienoic + polyenoic	3.20	5.35	24.31	22.63	23.59	21.53
total unsaturated	51.53	59.04	74.01	71.41	68.59	70.61

[a] As percentage of total fatty acids.

Table V. Summary of Fatty Acid Composition of the Total Phospholipids in Beef and Chicken Dark and White Meat[a]

fatty acid	beef with pigment	beef without pigment	dark meat with pigment	dark meat without pigment	white meat with pigment	white meat without pigment
% saturated	41.54	41.20	32.50	32.04	36.02	32.30
% monoenoic	33.53	38.93	29.18	28.78	27.37	31.83
% dienoic	11.88	11.45	26.32	23.91	17.96	22.45
% polyenoic	13.05	8.42	12.00	15.27	18.65	13.42
total unsaturated	58.46	58.80	67.50	67.96	63.90	67.70

[a] As percentage of total fatty acids.

Table VI. Mean TBA Numbers and Sensory Scores in Cooked Beef, Chicken Dark Meat, and Chicken White Meat (Four Replicates for Each Treatment)[a,b]

treatments	beef TBA no.	beef taste panel score	chicken dark meat TBA no.	chicken dark meat taste panel score	chicken white meat TBA no.	chicken white meat taste panel score
(A) cooked meat with pigment, no nitrite	1.93[e]	2.42[c]	11.19[e]	1.92[c]	9.52[e]	2.10[c]
(B) cooked meat with pigment plus nitrite	0.21[c]	4.42[d]	2.32[c]	3.84[d]	1.37[c]	4.77[e]
(C) cooked meat without pigment, no nitrite	0.61[d]	3.50[d]	4.20[d]	3.42[d]	4.41[d]	3.17[d]
(D) cooked meat without pigment plus nitrite	0.42[c,d]	4.31[d]	1.42[c]	4.50[d]	2.12[c]	4.15[d,e]

[a] Taste panel score was from 1 to 5, with 1 being pronounced WOF and 5 no WOF. [b] All numbers in same column for the the same characteristic followed by same superscript are not significant at $P < 0.05$.

only variable was the presence or absence of meat pigments.

The fatty acid composition of the triglycerides and phospholipids in meat with and without pigments is presented in Tables IV and V. The pattern of fatty acid composition in both the triglycerides and phospholipids is in good agreement with the data presented by Hornstein et al. (1961, 1967), Katz et al. (1966), and O'Keefe et al. (1968). Total unsaturation in the triglycerides (Table VI) and phospholipids (Table V) was considerably higher in both chicken dark meat and white meat than in beef. Furthermore, there were no consistent and significant differences in the fatty acid composition of tissues with and without pigments. Thus, any differences in the extent of lipid oxidation (TBA values) between experimental treatments should be related to the effect of pigments and/or nitrite.

Changes in TBA Values and Taste Panel Scores. Mean TBA numbers and the corresponding mean taste panel scores for cooked meat are presented in Table VI. Analyses of variance indicated that highly significant ($P < 0.001$) differences occurred among treatments for both TBA values and taste panel scores. Results showed that the samples containing pigments and without added nitrite (A) had the highest TBA values and the lowest taste panel ratings. The samples without pigments and without added nitrite (C) had significantly ($P < 0.01$) lower TBA values than the samples with pigments containing no added nitrite (A). Further taste panel scores were significantly ($P < 0.01$) higher for the former than the latter. Thus, the meat pigments appeared to contribute to the development of WOF in cooked meat.

Results showed that the removal of the meat pigments caused a twofold reduction in the TBA numbers of chicken white meat and a threefold decrease for beef and chicken

dark meat. These results further underline the importance of meat pigments in lipid oxidation. Addition of nitrite also reduced TBA values, with a ninefold, sevenfold, and fivefold reduction for beef, chicken white meat, and dark meat, respectively. Taste panel evaluation confirmed the beneficial effects of both removal of heme pigments and the addition of nitrite. However, addition of nitrite was significantly ($P < 0.05$) more beneficial as a means of controlling oxidized flavor in cooked meat than removal of meat pigments. These results support the contention that nitrite converts the pigments to the catalytically inactive form (Zipser et al., 1964). The effect of nitrite in protecting against autoxidation of lipids and, thus, in preventing the development of WOF is consistent with the results presented by Sato and Hegarty (1971) and Fooladi et al. (1979).

Significant ($P < 0.01$) correlation coefficients were found between TBA numbers and taste panel scores with "r" values of -0.74, -0.91, and -0.87 for beef, chicken dark meat, and chicken white meat, respectively. These values confirm the existence of a relationship between WOF and panel scores as reported by Zipser et al. (1964).

Role of Heme and Non-heme Iron as Prooxidants. The first stage of this study suggests that meat pigments are major prooxidants in the development of WOF in cooked meat. Thus, the second stage of the experiment was conducted to study the relative contribution of heme and non-heme iron as prooxidants of lipid oxidation in cooked meat. The mean TBA values obtained in the second stage are presented in Table VII. The TBA value on addition of total fresh meat pigment to the residue was 5.00. When total fresh meat pigment was treated with 2% EDTA, however, the TBA value dropped to 1.55. After the fresh meat pigment extract was heated to destroy the pigment and the filtrate added back to the residue, the

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Table VII. Role of Heme and Non-heme Iron on the Development of TBA Numbers in Cooked Beef[a,b]

treatment no.	preparation of experimental treatments	mean TBA no.
1	residue + total raw meat pigments	5.00
2	residue + total raw meat pigment (chelated)	1.55
3	residue + total cooked free meat pigment	4.35
4	residue + total cooked free meat pigment (chelated)	1.46
5	residue + H_2O_2 treated total meat pigment	6.02
6	residue + H_2O_2 treated meat pigment (chelated)	1.54

[a] Each experimental treatment consisted of 100 g of beef residue in addition to 50 mL of the concentrated extract. [b] EDTA was used to chelate the inorganic or free iron at a concentration of 2%.

Table VIII. Concentrations of Total Iron, Heme Iron, and Free Non-heme Iron in Treated and Untreated Meat Pigment Extract

experimental treatments	Fe^{2+}, $\mu g/g$ of meat
1. total iron in fresh meat pigment extract	20.64
2. non-heme iron in fresh meat pigment extract	1.80
3. heme iron in fresh meat pigment extract	18.84
4. total iron in cooked meat pigment filtrate	5.51
5. free non-heme iron in cooked pigment filtrate	4.18
6. total iron in H_2O_2 treated meat pigment extract	13.59
7. free iron in H_2O_2 treated meat pigment extract (chelated[a])	12.33

[a] Chelated by adding 2% EDTA.

TBA value was 4.35. On treating part of this filtrate with 2% EDTA and adding it back to the meat residue, the TBA value dropped to 1.46. Thus, results indicate that the chelated non-heme iron was unavailable and lipid oxidation was inhibited.

When the heme pigment was destroyed by treatment with 30% H_2O_2 and the filtrate was added back to the residue, the TBA number of the cooked meat was 6.02. This indicates that addition of non-heme iron increased the rate of lipid oxidation. After treating part of this filtrate with 2% EDTA and adding it back to the residue, the TBA number dropped to 1.54. The results reflect the effectiveness of chelating iron as a means of preventing lipid oxidation in meat.

The concentration of heme and non-heme iron in the different meat pigment extracts are presented in Table VIII. The concentration of total iron in the fresh meat pigment extract was 20.64 $\mu g/g$ of meat. The level of non-heme iron in the fresh meat pigment extract was 1.80 $\mu g/g$ of meat, while the amount of bound iron was 18.84 $\mu g/g$ of meat. Thus, the level of bound iron in the meat was slightly above 90% and the non-heme iron comprised only 8.72%.

When the total fresh meat pigment extract was heated to destroy the heme molecule and release the iron, the concentration of non-heme iron in the filtrate was 5.51 $\mu g/g$ of meat or about 27.0% of the total iron. Thus, results showed that cooking released a significant amount of the bound iron, which would account for the increased rate of lipid oxidation on adding the cooked filtrate back

to the meat residue (Table VII).

The concentration of non-heme iron in H_2O_2 treated fresh meat pigment extract after filtration was 13.59 $\mu g/g$ of meat. Thus, the H_2O_2 treatment released approximately 60.0% of the total iron in the pigment extract. The increased level of free iron would explain the high TBA value observed for the meat containing the H_2O_2-treated pigment extract (Table VII).

Results demonstrate that non-heme iron is the major prooxidant of lipid autoxidation in cooked meat and meat products. These results confirm the reports of Sato and Hegarty (1971) and Love and Pearson (1974), that non-heme iron, and not myoglobin, is the principal prooxidant in cooked meat. It is possible that copper ions could be a prooxidant in meat and play a role in WOF, since it would be extracted by water along with free iron and the pigments. However, Sato and Hegarty (1971) demonstrated that cupric salts actually inhibited WOF, apparently by the reaction of free radicals with cupric ions. Thus, non-heme iron appears to be the major prooxidant in development of WOF.

Results also demonstrated that non-heme iron is released from the heme pigments due to cooking, or by treatment with H_2O_2, thus accelerating lipid oxidation. These results verify the report by Haurowitz et al. (1941) that the prooxidant effect of hemin or hemoglobin on linoleic and linolenic acid is due to release of inorganic iron. Addition of 2% EDTA effectively chelated the non-heme iron and, thus, significantly reduced lipid oxidation in cooked meat.

LITERATURE CITED

Choudhury, R. B., Roy, B., Arnold, L. K., *J. Am. Oil Chem. Soc.* **37**, 87 (1960).
Folch, J. M., Lees, M., Stanley, G. H. S., *J. Biol. Chem.* **226**, 497 (1957).
Fooladi, M. H., Pearson, A. M., Coleman, T. H., Merkel, R. A., *Food Chem.* **5**, in press (1979).
Greene, B. E., *J. Food Sci.* **34**, 110 (1969).
Govindarajan, S., Hultin, H. O., Kotula, A. W., *J. Food Sci.* **42**, 571 (1977).
Haurowitz, F., Schwerin, P., Yensen, M. M., *J. Biol. Chem.* **140**, 353 (1941).
Hornstein, I., Crowe, P. F., Heimberg, M. J., *J. Food Sci.* **26**, 581 (1961).
Hornstein, I., Crowe, P. F., Hiner, R., *J. Food Sci.* **32**, 650 (1967).
Hougham, D., Watts, B. M., *Food Technol.* **12**, 681 (1958).
Igene, J. O., Pearson, A. M., *J. Food Sci.*, **44**, in press (1979).
Katz, M. A., Dugan, L. R., Jr., Dawson, L. E., *J. Food Sci.* **31**, 717 (1966).
Kwoh, T. L., *J. Am. Oil Chem. Soc.* **48**, 550 (1970).
Love, J. D., Pearson, A. M., *J. Agric. Food Chem.* **22**, 1032 (1974).
Maier, V. P., Tappel, A. L., *J. Am. Oil Chem. Soc.* **36**, 12 (1959).
Morrison, R., Smith, L. M., *J. Lipid Res.* **5**, 600 (1964).
O'Keefe, P. W., Wellington, G. H., Mattick, L. R., Stouffer, J. R., *J. Food Sci.* **33**, 188 (1968).
Sato, I., Hegarty, G. R., *J. Food Sci.* **36**, 1099 (1971).
Snedecor, G. W., Cochran, W. G., "Statistical Methods", 6th ed, Iowa State University Press, Ames, Iowa, 1973.
Tappel, A. L., *Food Res.* **17**, 550 (1952).
Tarladgis, B. G., Watts, B. M., Younathan, M. T., Dugan, L. R., Jr., *J. Am. Oil Chem. Soc.* **37**, 44 (1960).
Watts, B. M., *Adv. Food Res.* **5**, 1 (1954).
Wills, E. D., *Biochem. J.* **99**, 667 (1966).
Younathan, M. T., Watts, B. M., *Food Res.* **24**, 728 (1958).
Younathan, M. T., Watts, B. M., *J. Food Sci.* **24**, 734 (1959).

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Zipser, M. W., Watts, B. M., *Food Technol.* 16, 102 (1962).
Zipser, M. W., Kwon, T., Watts, B. M., *J. Food Sci.* 27, 138 (1964).

Effect of Pork Belly Composition and Nitrite Level on Nitrosamine Formation in Fried Bacon

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A study was conducted to determine the effect of compositional factors (fat, moisture, protein) on nitrosamine formation in bacon prepared from matched pairs of pork bellies cut into thirds. The compositional factors varied significantly ($p = 0.05$) from section to section within the same side but did not vary from side to side within the same section of matched pair. Both N-nitrosopyrrolidine and N-nitrosodimethylamine were most highly correlated with residual and added nitrite and to a lesser degree with the compositional factors.

N-Nitrosopyrrolidine (NPYR) (Crosby et al., 1972; Fazio et al., 1973) and to a lesser extent N-nitrosodimethylamine (NDMA) have been found consistently in fried bacon at the ppb level (Sen et al., 1973; Wasserman et al., 1978), while these and other nitrosamines have been found only sporadically in other cured meat products (Fiddler et al., 1975; Gough et al., 1977; Havery et al., 1976; Panalaks et al., 1974; Wasserman et al., 1972). The presence of nitrosamines in this product may be due primarily to the high cooking temperatures which would favor the reaction of residual nitrite with nitrosatable amine compounds. Several authors have demonstrated the importance of cooking temperature and method on the NPYR content of bacon (Herring, 1973; Pensabene et al., 1974; Wasserman et al., 1978). However, neither ham nor breakfast beef subjected to similar cooking conditions produces detectable concentrations of NPYR (Fiddler et al., 1974). This suggests that bacon may be unique in containing more readily nitrosatable precursor(s). Fiddler et al. (1974), Patterson et al. (1976), and Mottram et al. (1977) have associated NPYR formation with bacon adipose tissue and not with lean tissue. We have observed that bacon having a high fat to lean tissue ratio and yielding more rendered fat tended to have a higher concentration of NPYR than bacon having a lower fat to lean ratio. In our studies on bacon and nitrosamine formation, proper sampling has always been a problem because of the great variability of fat, moisture, and protein content of the green bellies used for processing. Stiffler et al. (1975) reported average lean differences as high as 10% at ten different anatomical positions. Schroder and Rust (1974) reported average fat contents ranging from 30 to 70% at 32 belly positions and concluded that there was as much compositional variation within the same belly as among different bellies. No significant difference in composition was observed between similar sections of paired bellies from the same carcass.

We are reporting here a new sampling scheme for investigating nitrosamine formation and for determining the effect of compositional factors on nitrosamine formation in fried bacon.

EXPERIMENTAL SECTION

Bacon Processing. Skinned matched pork bellies were purchased from a local supplier within 1 day of slaughter and stored for 1 week in a cooler at 1 °C. The bellies were cut into thirds (brisket, center, and flank sections) and pumped to approximately 10% of their green weight to achieve added target levels of 1.5% sodium chloride, 0.5% sugar, 0.3% sodium tripolyphosphate, and 200 ppm sodium nitrite (actual range 170–260 ppm). The pumped bellies were stored in polyethylene bags at 1 °C for 20–22 h, then processed in a smokehouse with the following schedule of increasing heat and controlled humidity: 1 h dry bulb (DB) 38 °C, wet bulb (WB) 0 °C; 1 h DB 50 °C, WB 0 °C; 3 h DB 57 °C, WB 47 °C. A medium to heavy smoke was introduced after 2 h of drying. The finished bacon reached an average internal temperature of 53 °C (128 °F) after 5 h. The bacon sections were placed in polyethylene bags and stored at 1 °C for 18 h.

Bacon Sampling and Frying. Each section of the belly was ground and thoroughly mixed three times through a $^1/_8$ in. plate prior to analyses. A 350-g representative sample of the comminuted bacon was fried for 6 min, with turning every 2 min, at a calibrated temperature of 177 °C (350 °F) in a preheated Presto Teflon-coated electric frying pan. Both the edible portion and rendered drippings were retained for nitrosamine analyses.

Bacon Analysis. *a. Nitrite.* Residual nitrite values were determined before frying by the Griess-Saltzman reaction in the procedure described by R. N. Fiddler (1977). The added nitrite values were calculated.

b. Fat, Moisture, Protein. Fat determinations were made by the Foss-let solvent extraction procedure described by Pettinati and Swift (1975). Moisture determinations followed the oven drying method (Official Methods of Analysis, 1975a), and protein analysis was carried out by the Kjeldahl procedure (Official Methods of Analysis, 1975b).

Nitrosamine Analysis. *a. Fried Bacon.* A 25-g fried bacon sample, to which 1 mL of N-nitrosomethylethylamine internal standard (0.5 μg/mL of CH_2Cl_2 solution)

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