with 60% (fresh weight basis) water. The seeded rice culture medium was kept for 7 days at 25 °C and 30 days at 12 °C. At the end of this period, the cultures were dried and ground to a fine mesh.

The dry culture (2.5 kg) was moistened with 30% water (20 mL of water/100 g of dry cultures); about 3 L of ethyl acetate was added to this moistened culture and kept at 5 °C for 24 h and filtered. The residue was once more extracted with 2 L of fresh ethyl acetate. The combined ethyl acetate was concentrated to a gum, which was redissolved in 600 mL of acetonitrile and partitioned against 600 mL of petroleum ether (bp 60–70 °C); the petroleum ether was discarded. The acetonitrile layer was concentrated and chromatographed on 800 g of silica gel (Davison 923, 100 mesh, activated at least 1 h at 110 °C, slurry packed in dichloromethane, eluting solvent: 20% ethyl acetate in dichloromethane → 100% ethyl acetate). The fractions were collected in 300-mL portions and were examined for zearalenone and its derivatives by TLC (Jackson et al., 1976). The fractions eluted with 2 L of 20% ethyl acetate contained mainly zearalenone. The fractions eluted with 3 L of ethyl acetate were enriched in F-5-4 and F-5-3 and those eluted with 3 L of 100% ethyl acetate had highly polar fluorescent components besides F-5-4 and F-5-3.

The fraction enriched in F-5-4 and F-5-3 was chromatographed on five preparative TLC plates developed three times in chloroform–absolute ethanol (97:3). The bands corresponding to F-5-4 and F-5-3 were removed and eluted with acetone; these components were successively rechromatographed on TLC to give 40 mg of F-5-4 (mp 168–168.5 °C) and 30 mg F-5-3 (mp 201–202 °C). Both compounds were identical with the authentic samples as determined by TLC, GC/MS, UV, and mass spectra.

The fraction containing components more polar than F-5-3 were chromatographed on four preparative TLC plates and were developed four times in chloroform–absolute ethanol (97:3). A fluorescent band which appeared just below F-5-3 was eluted with acetone; the evaporation of acetone gave approximately 40 mg of a yellow viscous residue. This residue was rechromatographed on preparative TLC plate to give approximately 15 mg of the material, which had ^{1}H NMR and UV spectrum similar to zearalenone. This material was further purified by TLC to yield 6 mg of pure sample. A ^{13}C NMR spectrum obtained on this compound was similar to that of F-5-3 and F-5-4. The compound was further resolved into two components at R_f 0.28 and 0.33, which were designated as F-5-1 (<1 mg) and F-5-2 (<2 mg: mp 168–169.5 °C). Anal. Calcd. for $C_{18}H_{22}O_5$: (F-5-1) 334.1415; found 334.1426; (F-5-2) 334.1415, found 334.1416. UV spectrum F-5-2 in MEOH: 235 (21036), 274 (9155), 336 (4493).

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Effect of Processing on the Amino Acid Composition and Nitrosamine Formation in Pork Belly Adipose Tissue

Ann M. Spinelli-Gugger,* Leon Lakritz, and Aaron E. Wasserman

The curing process does not alter the concentration of free amino acids in the adipose tissue of pork bellies. Frying of the adipose tissue leads to a decrease in the level of free amino acids; greater reductions were seen in the processed vs. untreated tissue. Analysis for nitrosamines in the fried samples indicate that dimethylnitrosamine and nitrosopyrrolidine formation is independent of free amino acid concentration. A study of the lean, adipose and intact tissue of country cured bacon indicates that frying generates up to a tenfold increase in free amino acids in the lean and intact tissue, while comparatively negligible changes are noted in the adipose.

The potential human health hazard posed by nitrosamines (NAs) was recognized as early as 1954 by Barnes and Magee. NAs are produced by the acid-catalyzed reaction of nitrite or nitrogen oxides with certain nitrogen-containing compounds. Amino acids, amines, and amides are examples of these compounds that are present as natural constituents of meats and other foodstuffs (Lijinsky et al., 1970; Ender and Ceh, 1971; Bills et al., 1973; Huxel et al., 1974). The formation of NAs has been reported in model systems in which amino acids are heated with sodium nitrite at elevated temperatures (~170 °C) similar to those attained when frying bacon (Ender and Ceh, 1971; Bills et al., 1973; Huxel et al., 1974; Gray and Dugan, 1973; Coleman, 1978).

Traditionally, nitrite and to a lesser extent nitrate have been used to prepare cured meats that are shelf-stable and possess desirable color and flavor characteristics. Although NAs are not found consistently in all cured meat products

Eastern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

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in which nitrite is an additive, nitrosopyrrolidine (NPYR) has been confirmed repeatedly in fried bacon (Sen et al., 1973; Fazio et al., 1973; Fiddler et al., 1974; Patterson et al., 1976; Havery et al., 1976; Gray, 1976). The formation of NPYR has been thought to occur by either of two mechanisms: decarboxylation of proline to pyrrolidine which is then nitrosated or the initial formation of nitrosoproline followed by decarboxylation. Recent studies, however, indicate that NPYR forms almost exclusively in the bacon adipose tissue rather than in the lean tissue where most amino acids are found (Fiddler et al., 1974; Mottram et al., 1977). Amino acid data, however, on pork belly adipose tissue are limited.

We determined amino acid concentrations of adipose tissue in matched pairs of processed and untreated bellies before and after frying and nitrosamine values from the corresponding samples after heating them with excess nitrite to ensure maximum NA formation. We excluded ascorbate from the cure solution because it reacts competitively with nitrite, which could reduce NA formation. We also analyzed "Country Cured" bacon, a commercial product, which contains no ascorbate and, therefore, closely resembles the bacon used in this study.

EXPERIMENTAL SECTION

a. Bacon Processing. Matched pairs of freshly skinned pork bellies were purchased from a local packing plant within 3 days of slaughter. One side of each pair was stored at 4 °C; the other side was pumped to 110% of green weight with a cure pickle consisting of 787 mL of H_2O, 147 g of NaCl, 50 g of sugar, 30 g of sodium tripolyphosphate, and 1.6 g of $NaNO_2$. The final target levels achieved prior to smoking were 1.5% sodium chloride, 0.5% sugar, 0.3% sodium tripolyphosphate, and 120 ppm sodium nitrite. The bellies were then stored in polyethylene bags at 1 °C for 18–24 h prior to being processed into bacon. The smokehouse schedule was 1 h at 48.9 °C (120 °F) and 3–4 h at 58.3 °C (137 °F) at 50% relative humidity, with a medium level of smoke produced from hardwood sawdust.

b. Sample Preparation. Adipose and lean tissues from entire bellies were separated manually from green bellies, laboratory processed bacon, and commerical dry-cured bacon. Separated and intact tissues were ground twice and mixed thoroughly to attain sample homogeneity and then stored at −20 °C until used. All samples that were heated had 1000 ppm $NaNO_2$ added; they were then fried in a preheated Presto Teflon-coated electric frying pan (Model PC4AT) for 6 min at 177 °C (350 °F). Intact and adipose tissues were cooked normally, but in order to attain the same temperatures the lean tissue was fried in a hydrogenated vegetable oil.

METHODS OF ANALYSIS

a. Amino Acids. The extraction procedure used was similar to that described by Lakritz et al. (1974). The acid–neutral amino acids were separated on a Spherix XX8-60-O resin in a Phoenix Model M-7800 amino acid analyzer, by the accelerated system of Spackman et al. (1958), with sodium citrate buffers.

b. Volatile Nitrosamines. The NAs in the cooked samples were determined by the isolation procedure of Fazio et al. (1971) as modified by Pensabene et al. (1974), with a silica gel acidified Florisil chromatographic column. Samples were quantitated by a gas–liquid chromatograph interfaced with a thermal energy analyzer (Model 502) under conditions similar to those reported by Fine et al. (1975). NAs were confirmed by a GLC–high-resolution mass spectrometer (Model DuPont 21-492) (Pensabene et al., 1974).

Table I. Amino Acid Composition of Pork Belly Adipose Tissue

amino acids	concn,[a] μM/100 g untreated	processed
Asp	2.3 ± 0.5	2.5 ± 0.6
Thr-Ser[b]	44.9 ± 5.1	57.1 ± 4.8
Glu	11.0 ± 3.3	27.8 ± 15.8
Pro	8.0 ± 0.8	12.1 ± 1.6
Gly	40.6 ± 4.8	57.2 ± 8.1
Ala	46.2 ± 12.2	41.3 ± 8.1
Val	11.1 ± 1.8	12.8 ± 3.2
Met	1.9 ± 0.6	1.6 ± 0.5
Ile	4.3 ± 0.6	5.6 ± 1.6
Leu	9.4 ± 2.0	9.6 ± 2.8
Tyr	3.1 ± 2.0	2.6 ± 1.5
Phe	1.5 ± 0.8	2.4 ± 1.4

[a] Mean and standard deviation values represent five matched pairs of bellies. [b] Chromatographically unresolved.

Table II. Amino Acid Composition of Raw and Fried Pork Belly Adipose Tissue

amino acids	concn,[a] μM/100 g untreated raw	untreated fried	processed raw	processed fried
Asp	2.9 ± 0.6	0.8 ± 0.2	3.1 ± 0.8	0.9 ± 0.4
Thr	37.3 ± 4.6	4.0 ± 0.8	42.9 ± 4.4	1.1 ± 0.5
Ser	25.7 ± 2.8	8.3 ± 0.8	30.3 ± 1.9	1.7 ± 0.2
Glu	23.6 ± 5.2	3.5 ± 0.9	25.6 ± 6.3	1.3 ± 0.3
Pro	9.4 ± 2.0	7.8 ± 1.0	13.0 ± 1.0	2.9 ± 0.7
Gly	35.0 ± 3.8	25.2 ± 13.8	53.2 ± 5.7	7.4 ± 2.0
Ala	55.7 ± 1.9	68.3 ± 7.0	58.5 ± 2.9	17.7 ± 1.1
Val	10.4 ± 3.1	6.2 ± 0.4	12.9 ± 1.3	3.0 ± 0.8
Met	3.0 ± 0.6	3.0 ± 0.5	3.3 ± 0.5	0.5 ± 0.2
Ile	5.2 ± 1.3	3.6 ± 2.2	5.1 ± 1.0	3.2 ± 2.3
Leu	12.0 ± 2.2	9.0 ± 1.2	9.8 ± 3.9	1.3 ± 0.8
Tyr	10.4 ± 0.9	3.6 ± 1.4	10.8 ± 0.9	0.8 ± 0.3
Phe	5.2 ± 1.1	4.6 ± 1.4	6.6 ± 1.3	0.2 ± 0.1

[a] Mean and standard deviation values represent three matched pairs of bellies.

RESULTS AND DISCUSSION

The amino acid composition of the adipose tissue of five matched pairs of bacon bellies, one side processed and the other left untreated, is shown in Table I. Comparison tests indicated significant increases ($P < 0.05$) in the concentrations of proline (52%) and glycine (41%) occur as a result of processing. Although there appears to be a major increase in glutamic acid concentration, this is the result of only one set among the five pairs. Minor variations occur in the concentrations of the other free amino acids. When bacon is fried, the high temperature (in the order of 350 °F) could either increase the free amino acid pool by protein degradation or cause loss in the existing pool as a result of thermal reactions. The adipose tissue from three additional pairs of bellies were analyzed before and after being fried; one belly of each pair was processed.

The concentration of amino acids in the adipose tissue of raw and processed bellies prior to being fried (Table II) are generally comparable to those reported in Table I, with the exception of glutamic acid concentration. After being fried, the green adipose tissues register losses of amino acids as high as 85 and 89% for glutamic acid and threonine, respectively, but for the most part, decreases are more moderate and in the order of 25–40%. Frying the processed samples, however, results in decreases in amino acid concentration of 70% or more for all amino acids except isoleucine. Differences between the amino acid concentrations in the processed and untreated samples are considerably greater after frying than before, emphasizing the

Table III. Proline, Glycine, and Nitrosamines from Adipose Tissue of Green and Processed Pork Bellies[a]

matched pair	treatment	Pro,[b] ppm	NPYR,[c] ppb	Gly, ppm	NDMA,[c] ppb
1	none	9.7	3	27.1	17
	proc	13.6	8	42.2	41
2	none	8.9	8	35.3	14
	proc	13.0	45	41.0	112
3	none	8.1	8	32.9	16
	proc	12.0	3	38.9	16
4	none	8.7	20	29.6	32
	proc	15.9	20	53.4	nd[d]
5	none	10.6	nd	27.4	nd
	proc	16.0	17	36.5	12
6	none	8.3	56	23.8	44
	proc	13.7	68	39.6	68
7	none	12.8	55	25.7	32
	proc	15.4	55	35.9	21
8	none	11.5	84	29.4	36
	proc	15.9	76	44.3	60

[a] All NA values confirmed by mass spectrometry. Values of matched pairs 1-5 represent results obtained in conjunction with Table I and pairs 6-8 correspond to results in Table II. [b] Amino acid available in tissue prior to frying. [c] NAs after frying. Nitrite was added to all samples prior to frying. [d] None detected.

effect of cure salts and heat on the stability of the amino acids in these adipose tissues. Proline, one of the amino acids suggested as a precursor for NPYR, presumably forms from the adipose tissue of bacon on frying. The concentration of proline, as well as that of glycine, increases significantly following processing, suggesting their formation as a result of the decomposition of collagen during processing. The proline concentrations in the adipose tissue of all eight pairs of bellies, processed and untreated, and the NPYR concentrations formed during frying with 100 mg of $NaNO_2$ are shown in Table III. There is no correlation between the concentration of proline in the unfried material and the concentration of NPYR formed on heating. The addition of nitrite to the untreated tissue led to the formation, in some instances, of as much as or more NPYR than appeared in the processed matched sample. It is interesting to note that the last three matched pairs of bellies (6-8 in Table III), which were used in the experiment detailed in Table II, produced very high levels of NPYR. No known variations in experimental procedure could have produced the additional nitrosamine. Nitrosodimethylamine was also observed in most of the fried adipose tissue samples. Since this nitrosamine has been detected during the pyrolysis of glycine (Ender and Ceh, 1971), the relationship of glycine to NDMA is also shown in Table III. The concentrations of NDMA were highly variable, ranging from none detected to 44 ppb in the untreated tissue and to 112 ppb in processed tissue. There was no correlation between the concentration of glycine in the tissue and the amount of NDMA nor between the concentrations of NDMA and NPYR formed in each of the samples. In general, however, the processed adipose tissue appeared to give rise to higher concentrations of NAs than did the untreated tissues, possibly because they were exposed to greater concentrations of sodium nitrite, 120 ppm in the cure mix plus 1000 ppm prior to being fried, whereas untreated tissues received only 1000 ppm sodium nitrite.

We also analyzed commercially produced "Country Cured" bacons because they are prepared without sodium ascorbate and thereby more closely resemble the bacons we studied in this investigation. Amino acid concentrations were determined after the intact, lean, and adipose tissues were fried, with the results summarized in Table IV. Comparison of these data with the results from the analysis of 18 raw untreated bellies (Table V) shows that raw adipose tissue accounts for much less of the free amino acid content than does either the raw intact or lean tissue. As a result of frying, the amino acid concentration in the intact and lean tissue was many-fold greater than in the adipose tissue.

Table IV. Amino Acid Concentrations in Fried Commercial "Country Cured" Bacon

	concn,[a] μM/100 g tissue		
amino acid	intact	lean	adipose
Asp	70.8	142.0	7.8
Thr	116.7	262.9	9.3
Ser	183.8	612.8	17.3
Glu	227.9	295.2	9.2
Pro	119.0	280.4	1.0
Gly	251.8	540.4	32.8
Ala	382.2	596.8	34.9
Val	134.0	316.8	15.6
Met	26.2	89.4	1.4
Ile	79.1	220.2	5.6
Leu	141.1	369.4	7.3
Tyr	51.4	129.2	1.7
Phe	66.4	137.4	2.5

[a] Average value of three bacons.

Table V. Amino Acid Concentrations in Untreated Pork Bellies

	concn,[a] μM/100 g tissue		
amino acid	intact	lean	adipose
Asp	18.9	26.3	2.5
Thr	24.3	26.7	27.2
Ser	36.0	31.7	25.7
Glu	26.5	33.5	10.6
Pro	14.9	23.9	6.9
Gly	109.0	148.4	34.8
Ala	170.9	199.1	46.2
Val	17.6	167.1	8.1
Met	4.2	5.7	1.7
Ile	9.9	14.5	3.7
Leu	16.9	23.0	8.2
Tyr	7.2	11.1	5.4
Phe	8.1	11.1	3.1

[a] Average value of 18 untreated bellies.

From the data presented in this paper, statistical analysis (comparison tests and analysis of variance techniques) of the differences in amino acid content before and after frying and NA concentration found after frying showed no significant correlation. Contrary to what has recently been reported (Bharucha et al., 1979; Coleman, 1978; and Hwang and Rosen, 1976), our data indicate that the NDMA and NPYR contents of fried bacon are independent of the concentration of free amino acids. Nitrosamine concentration was not significantly affected when the amino acid source, including proline, was decreased nor when the availability of free amino acids increased significantly. In conclusion, although free amino acids may play some role in NA formation in fried bacon, we think that they are not the limiting factor and that other substances and/or conditions present in the adipose tissue are responsible for the quantities of NAs formed in bacon.

(Precautions should be exercised in the handling of nitrosamines since they are potential carcinogens.)

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Coniferyl and Sinapyl Alcohols: Major Phenylpropanoids Released in Hot Water Extracts of Tobacco and Alfalfa

Roger A. Andersen,* Thomas H. Vaughn, and Michael J. Kasperbauer

Coniferyl alcohol and sinapyl alcohol were identified in water and methanol extracts of tobacco stalk and alfalfa stem at molar concentration ratios near unity, but they were not detected in chloroform or acetone extracts. Water extracts contained higher levels of these alcohols than methanol extracts. Our interpretation was that these differential solvent effects were caused by different capacities of the solvents to hydrolyze covalently bound coniferyl and sinapyl alcohols in the plant lignin or carbohydrate matrix. The amount of each alcohol extracted from tobacco stalk was dependent upon the extraction time. The identification of coniferyl and sinapyl alcohols was based on high-performance liquid chromatography capacity factor (k') values and gas chromatography retention times of Me_3Si derivatives. Further confirmation was obtained by gas chromatography–mass spectrometry. Synthesis of coniferyl and sinapyl alcohols was accomplished by reduction of the corresponding acid chlorides to aldehydes and alcohols with lithium tri-*tert*-butoxyaluminohydride.

Metabolites of *p*-coumaric acid in higher plants are the source of most phenols, which include flavanoids, polyphenols, coumarins, tannin and lignin precursors, tannins, and lignin (Neish, 1964). Most tobacco plant parts contain soluble phenols as chlorogenic acid isomers, rutin, scopoletin, scopolin, and esculetin (Sheen, 1969; Vaughn and Andersen, 1973) in addition to insoluble phenols such as lignin (Andersen and Litton, 1975). Quantities of total soluble phenols determined in tobacco were not completely accounted for by the summations of individual phenolic constituents (DHEW Publication No. (NIH) 77-1280, 1977). Unidentified soluble tannins or phenylpropanoids may account for some of this discrepancy. There has been no report of the presence in tobacco of any of the postulated monomeric lignin precursors (Table I), namely, *p*-coumaryl, coniferyl, and sinapyl alcohols, the aldehyde and acid congeners, or their respective phenolic glycosides (Freudenberg, 1965; Freudenberg, 1966). Coniferyl alcohol, however, was recently identified in the phenolic fraction of condensate derived from the smoke of cigarettes made with either flue-cured tobacco leaf midrib or lamina; its concentrations were twice as high in midrib cigarette tobacco condensate as in lamina condensate (Ishiguro et al., 1976).

Recent statistical evidence based on correlations of the chemical composition of experimental cigarettes and the health-related biological activity of their derived smoke suggested that high levels of soluble phenols in tobacco leaf are undesirable (DHEW Publication No. (NIH) 76-1111, 1976;, DHEW Publication No. (NIH) 77-1280, 1977; USDA Technical Bulletin No. 1551, 1977). New smoking materials may contain greater proportions of tobacco from plant tissues that have more lignified cell walls than the conventional materials of the past (Atkinson, 1961; Andersen et al., 1979). Therefore, these materials may contain more phenolic lignin-precursor compounds. In this paper we

Agricultural Research, Science and Education Administration, U.S. Department of Agriculture and Department of Agronomy, University of Kentucky, Lexington, Kentucky 40546.

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