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Interactions of Some Wood Smoke Components with ϵ-Amino Groups in Proteins

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Interactions of some previously identified wood smoke components with meat and pure proteins were investigated. Uncured lean beef sirloin strips, exposed to wood smoke for 10 hr, lost 44% of the available lysine initially present. Heating in air (65°, 10 hr) caused a 15% loss of available lysine. Beef homogenate, treated with acidic, phenolic, or neutral fractions of smoke condensate, lost 14, 38, and 45% available lysine, respectively. A model system consisting of a soluble protein, bovine serum albumin (BSA), was treated with pure smoke components and the degree of interaction was determined spectrophotometrically by measuring the extent to which the protein reacted with dinitrobenzyl sulfonate. The reduction in available ϵ-amino groups observed when BSA was treated with sinapaldehyde and coniferaldehyde was comparable to that observed for some previously known active aldehydes (formaldehyde, glyoxal, pyruvaldehyde, and furfural). No significant interaction was detected when BSA was treated with phenol, cyclotene, eugenol, or syringol. A variety of colors, ranging from yellow to red, was produced by treating casein with aldehydes and phenolic aldehydes.

The preservative effects of wood smoke on foods result from partial surface dehydration and from deposition of compounds with antimicrobial and antioxidant activity. In current commercial practice in the United States, smoking is employed primarily for color and flavor, but significant extension of shelf-life may result from the smoke components added to the products.

In view of current concern about the nutritional value of processed foods and about chemicals added to foods, it is important not only to know the identities and amounts of smoke components consumed, but also the nature of reaction products formed during exposure of foods to smoke. Many wood smoke components have been identified (Fiddler *et al.*, 1966, 1967, 1970a,b; Hamid and Saffle, 1965; Hoff and Kapsalopoulou, 1964; Jahnsen, 1961; Love and Bratzler, 1966; Lustre and Issenberg, 1969; Porter *et al.*, 1964; Ruiter, 1970), some of these were identified in smoked food products (Bratzler *et al.*, 1969; Howard *et al.*, 1966; Lustre and Issenberg, 1970; Shewan, 1953; Tucker, 1942), but little attention has been given to chemical reactions in smoked foods or to the reaction products.

Knowledge of chemical reactions during the smoking process can provide background necessary for evaluation of the functional and nutritional properties of protein in smoked foods. Such studies may also reveal the presence of reactive compounds with potential commercial value as coloring reagents and antimicrobial compounds.

Reactions of aldehydes with free amino groups are well known. Loss of available lysine in meats treated with wood smoke was attributed to reactions of formaldehyde with the ϵ-amino group of lysine (Dvorak and Vognarova, 1965; Inagami and Horii, 1966). Ruiter (1970) suggested that glycolic aldehyde and pyruvaldehyde can react with protein. The reaction between glycolic aldehyde and aminoethanol was examined in detail (Ruiter, 1971). The present investigation undertakes to determine the extent to which some specific wood smoke components react with ϵ-amino groups of lysine in protein and to establish the significance of these components in causing loss of available lysine during smoking. We wished to determine whether interaction of proteins with coniferaldehyde and sinapaldehyde, major phenolic components of wood smoke condensates, could account for inability to recover these compounds from smoked food products (King, 1970; Lustre and Issenberg, 1970).

EXPERIMENTAL SECTION

Smoking of Samples. The glass smoke generator and the smokehouse were described previously (Lustre and Issenberg, 1970). Fresh lean beef sirloin was purchased locally. Samples to be smoked were sliced as strips 2 to 3 mm thick, 1 to 2 cm wide, and 15 to 20 cm long. Individual strips were held at one end with straightened paper clips and suspended from a rack located at the top of the smokehouse. They were hung in four rows, 5 cm apart, and samples within a row were separated by 6 to 10 cm. This arrangement maximized the

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Table I. Available Lysine Loss in Smoked Lean Beef Sirloin

Sample	Available lysine, g per 16 g N	% Loss
Untreated	7.51	0
Heated (65°, 10 hr)	6.37	15.2
Smoked (65°, 10 hr)	4.17	44.5

Table II. Available Lysine Loss in Lean Beef Sirloin Suspension Treated with Smoke Condensate Fractions

Sample	Available lysine, g per 16 g N	% Loss
Untreated	7.51	0
Acidic fraction	6.46	14
Phenolic fraction	4.66	38
Neutral fraction	4.13	45

surface area of the sample exposed to smoke. Total smoking time was 10 hr.

An unsmoked meat blank was prepared in the same manner and exposed to experimental conditions identical to those used in smoking except for the smoke. Samples were exposed to an air flow of 500 to 600 ml per minute at 65° for 10 hr. The air passed through a bed of sawdust in the smoke generator before entering the smokehouse.

Available Lysine Determination. Fifty-gram samples of beef sirloin were ground twice in a meat grinder, blended with 200 ml of distilled water in a Waring Blendor for 5 min, and after addition of 300 ml of distilled water, the mixture was homogenized (Virtis "45" tissue homogenizer) for 10 min at low speed. Large particles were filtered out with a Buchner funnel. Available lysine in untreated, heated, and smoked beef samples were determined by Carpenter's (1960) method. Total nitrogen (A.O.A.C., 1970) was determined in all meat samples.

Treatment of Beef Suspension with Smoke Fractions. Smoke condensates were collected and separated into phenolic, acidic, and neutral fractions by the method described by Lustre and Issenberg (1969). Lean beef sirloin homogenate was prepared as described above. Fifty milliliters of beef suspension, containing approximately 1 g of meat protein, was treated with approximately 200 mg of the phenolic, acidic, and neutral fractions, respectively. The mixtures were shaken at 37° for 24 hr. Available lysine in these mixtures were determined after treatment.

Reaction of Smoke Components with ε-Amino Groups of Bovine Serum Albumin (BSA). Bovine serum albumin (50 mg/ml) in 0.02 *M* pH 6.0 phosphate buffer was treated with 200 mg of each test compound. After the mixture was shaken at 37° for 24 hr, unreacted test compound was removed by dialyzing the reaction mixture against running distilled water for 24 hr. Potassium carbonate (50 mg) and 40 mg of dinitrobenzyl sulfonate sodium salt were then added. The mixture was shaken at 37° for 24 hr and then dialyzed against running distilled water until free dinitrobenzyl sulfonate was removed (24 hr). During these treatments, all containers were shielded from light with aluminum foil.

The DNP–protein solutions were then diluted with 0.1 *N* NaOH; absorbance was measured at 290 and 360 nm. The number of DNP groups bonded to ε-amino groups per bovine serum albumin molecule was calculated (Eisen *et al.*, 1954).

Smoke components found to be reactive in the experiment described above were added (5 μmol in 5 to 10 μl of 15% methanol) to 0.5 mg of BSA in 50 μl of 0.02 *M* phosphate buffer, pH 6.0, in a glass tube. The reaction was stopped after 3- to 45-hr periods by TCA precipitation of the protein. The precipitate was washed with ethanol and acetone and dissolved in 0.2 ml of 8% $NaHCO_3$. Fluorodinitrobenzene solution (80 μl in 0.1 ml of ethanol) was added and the mixture was shaken for 2 hr. After addition of 0.5 ml of 8.1 *N* HCl, the tube was sealed and the mixture was hydrolyzed at 110° for 24 hr. DNP–lysine was then extracted and determined spectrophotometrically (Carpenter, 1960).

Treatment of Casein with Smoke Components. Casein (Nutritional Biochemicals, Cleveland, Ohio) was treated with sinapaldehyde, coniferaldehyde, pyruvaldehyde, glyoxal, furfural, and formaldehyde. Six millimoles of each test compound was added to 3 g of suspended casein in 10 ml of 15% aqueous methanol. The mixtures were air dried in crystallizing dishes at room temperature for 24 hr. An identical series of mixtures was dried at 65° and 20 Torr for 3 hr.

RESULTS AND DISCUSSION

Dvorak and Vognarova (1965) suggested that short-time smoking had no effect on lysine availability but that loss was evident after exposure to smoke for several hours or days. Available lysine loss was influenced by smoking time, smoking temperature, storage time, and water activity. Up to 43.7% loss of available lysine was observed by Dvorak and Vognarova (1965) in Hungarian salami with 15% water content. This product was smoked with cold smoke for 2 days and stored at 20° for 1 year.

In our studies, there was 44.5% loss of available lysine in 2- to 3-mm lean beef sirloin slices smoked for 10 hr at 65° (see Table I). In the unsmoked heat-treated sample, 15.2% loss was observed. The extent of available lysine loss in the smoked samples suggested that reactions between ε-amino groups of proteins and smoke components were likely. The effects of treating beef homogenate with smoke fractions (Table II) showed that the phenolic and neutral fractions contain components which react with ε-amino groups. The neutral fraction was the most active, but significant activity was present in the phenolic fraction. The terms "neutral fraction," "phenolic fraction," and "acidic fraction" only describe their general nature; procedures employed did not provide quantitative separation into specific compound classes. Many components were expected to be present in more than one fraction. It was necessary to evaluate reactivity of individual components of each fraction to ensure that the effects observed were not caused by a single component present in both the phenolic and neutral fractions.

Carpenter's (1960) method of available lysine determination was a laborious and time-consuming procedure. Total acid hydrolysis of DNP–protein and isolation of ε-DNP–lysine were necessary. Use of the BSA model system significantly simplified these studies. Under the reaction conditions described, tyrosine hydroxyl groups did not react with DNP (Williams and Chase, 1967). The sulfhydryl group of cysteine might be substituted by DNP, but *S*-DNP–cysteine residues are unstable at the alkaline pH at which the reaction was carried out; they yield dehydroalanyl residues plus free 2,4-dinitrothiophenylate (Sokolovsky *et al.*, 1964). Free α-NH_2 groups of N-terminal residues will react with DNP, but they are few relative to the lysine residues in BSA. Therefore, the BSA model, employing mild and easily controlled conditions, provides a screening system for reliable and specific measurement of interactions of test compounds with ε-amino groups. Unreacted reagent was readily removed from the derivatized proteins by dialysis. The exact DNP/BSA ratio depends on DNP concentration and reaction time (Williams and Chase, 1967), but the system should yield valid measures of relative reactivity under the standard conditions.

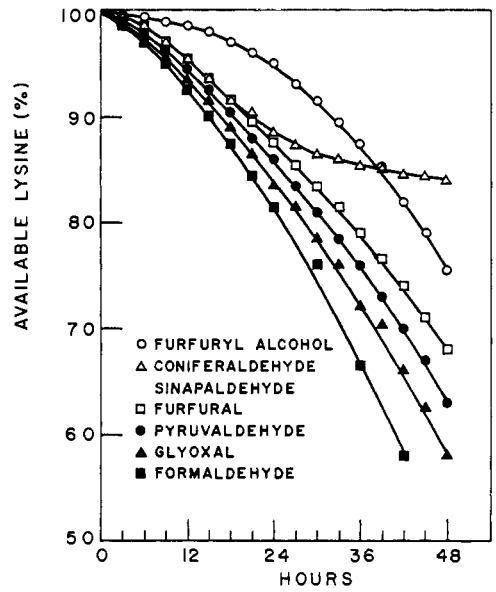

Figure 1. Decrease of available lysine in bovine serum albumin treated with selected smoke components

Results obtained with the aqueous BSA model are shown in Table III; formaldehyde, glyoxal, pyruvaldehyde, furfural, furfuryl alcohol, sinapaldehyde, and coniferaldehyde significantly reduced the number of ε-amino groups available for reaction with DNP.

We recognized that kinetics of the reactions leading to loss of ε-amino groups in smoked meats is likely to be complex. Reaction rates will be influenced by temperature, pH, and water activity, all of which change continuously during the smoking process. The model system selected, aqueous BSA at 37° and pH 6.0, provided information which is applicable only to these specific conditions. The curves shown in Figure 1 illustrate the complexity of the reactions even in the simplified model system. Of the compounds tested, formaldehyde exhibited the maximum reaction rate. Coniferaldehyde and sinapaldehyde reacted at a lesser rate initially and, after 24 hr, the reaction appeared to stop. No conclusions can be drawn from these data regarding mechanisms of reactions or relative rates under actual smoking conditions. A more realistic experimental model which provides for variation of water activity and pH must be developed. These results do support the suggestion (Dvorak and Vognarova, 1965) that formaldehyde might be the most active smoke component in reactions with the ε-amino group of lysine.

Though formaldehyde reacts rapidly with proteins, it is unlikely that the reaction products contribute significantly to the color of smoked foods. Colors produced by treating casein with selected smoke components are listed in Table IV. No pigment was produced in formaldehyde-treated casein, while a range of colored products was formed in samples treated with the other aldehydes listed. The contributions of these pigments to typical colors of smoked foods remain to be determined.

Table III. Binding of Dinitrophenyl Groups by ε-Amino Groups of Bovine Serum Albumin (BSA) after Treatment with Selected Smoke Components

Sample	mol of DNP/mol of BSA
Untreated BSA	17.0
Phenol	17.0
Cyclotene	16.0
Eugenol	16.0
Syringol	16.5
Formaldehyde	5.0
Glyoxal	6.0
Pyruvaldehyde	7.5
Furfural	8.0
Furfuryl alcohol	11.0
Sinapaldehyde	8.0
Coniferaldehyde	8.0

Table IV. Colors Produced by Treatment of Casein with Selected Wood Smoke Components

Smoke component	Color[a]
Untreated casein	White
Formaldehyde	White
Glyoxal	Yellowish brown
Pyruvaldehyde	Light brown
Furfural	Brown
Coniferaldehyde	Yellow
Sinapaldehyde	Orange

[a] After drying at room temperature for 24 hr or at 65° and 20 Torr for 3 hr; both drying methods were used for each sample.

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