Major Volatile Components of the Basic Fraction of Hydrolyzed Soy Protein

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Interest in the use of hydrolyzed vegetable protein in many food products has led to the need for more basic knowledge of the types of compounds present. The present study shows that alkylated pyrazines are the major compounds of significance in a volatile basic fraction of hydrolyzed soy protein. Via gas chromatography and mass spectroscopy, 10 pyrazines were positively identified and one was tentatively identified. Also positively identified were p- and m-cresol, 5-methyl furfural, benzaldehyde, and o-methoxy phenol. The mechanisms for the formation of pyrazines is discussed, along with the significance of the material in the aroma of the hydrolyzed soy protein.

Vegetable protein hydrolyzates have become an important commodity in fabricated food products. Because of this, there is a great need for fundamental information about the formation of aroma and the stabilization of the chemical components of nutritive value during the hydrolysis process. A study by Manley and Fagerson (1970) investigated the volatile acid and neutral components of the hydrolyzed materials. That investigation identified many major volatile components; however volatile material found in the basic extracts were also considered important in the overall aroma.

The purpose of the present study was to identify the major volatile components in these basic extracts of hydrolyzed soybean flour.

EXPERIMENTAL

The following method was devised to insure a highly efficient extraction of the basic material. A 500-g sample of spray-dried hydrolyzed soy protein prepared as outlined by Manley and Fagerson (1970) was placed into a distillation flask where one-half of the volume (about 500 ml) of water was distilled. This distillate, with a pH of about 4.5, had 5% HCl added to it until the final pH was below 1.0. The mixture was then saturated with NaCl and extracted with three volumes (50 ml) of dichloromethane. The solvent and acid material contained in it were discarded. The aqueous solution had 5% NaOH added until the pH of the mixture was between 8 and 9. This was subjected to extraction with three volumes (50 ml) of dichloromethane. The extract was then dried over anhydrous sodium sulfate and the solvent stripped under a flow of nitrogen. This material was distilled at a temperature of 50° C and a pressure of 1μ of Hg onto a liquid N_2-cooled cold finger. The material was removed from the cold finger by repeated washings with dichloromethane and considered to be the volatiles of the basic fraction.

Mass Spectroscopy. For analysis, a 1-μl sample of the concentrated ether extract was taken. Mass spectra were obtained using a coupled gas chromatograph (Varian Aerograph Model 1200)—mass spectrometer (Hitachi Perkin Elmer Model RMU-6A) unit with a capillary column, 50-ft long support-coated open tubular (SCOT) column 0.02-in. i. d. coated with Carbowax 20M (K20M).

The column effluent was split, with nearly equal portions being directed to the flame ionization detector and to the mass spectrometer. A Watson-Biemann (Watson and Biemann, 1965) helium separator was used between the gas chromatograph and the mass spectrometer. The operating parameters were as follows. Gas chromatograph: Flow rate—helium carrier gas at 15 ml/min; Program—100° C for 5 min, 100° C to 180° C at 6° C/min; Injection block temperature—275° C. Mass spectrometer: Inlet temperature—200° C; Ion source pressure—8×10^{-6} mm; Ion source temperature—250° C; Acceleration voltage—2.5 KV; Target current—100 A; Electron multiplier voltage—2.5 KV; Scan speed—8 sec $(m/e$ 12-250); Exit slit—0.25 mm.

Retention Data. The retention times of the known compounds and unknown sample were recorded by injection on a Perkin-Elmer 900 gas chromatograph, using the following columns for the various fractions.

A 50-ft SCOT column, 0.02 in. i.d., coated with Carbowax 20M (K20M).

A capillary column, 200 ft long, 0.02-in. i.d., and coated with diethylene glycol succinate (DEGS) and H_3PO_4 (2%).

Reference Compounds. The following compounds were synthesized by the method of van Praag et al. (1968): pyrazine; 2-methyl pyrazine; 2,5-dimethyl pyrazine; 2,6-dimethyl pyrazine; 2,3-dimethyl pyrazine; 2,3,5-trimethyl pyrazine; 2-ethyl pyrazine; 2-ethyl-5-methyl pyrazine; 2-ethyl-3-methyl pyrazine; 2-ethyl-3,5-dimethyl pyrazine; 2-ethyl-3,6-dimethyl pyrazine; 2-ethyl-3,5,6-trimethyl pyrazine. The production of these materials involved the boiling under reflux for 2 hr of D-glucose and L-rhamnose (100 g) with ammonium hydroxide (28%, 40 ml) and water (100 g).

The material was partially purified by distillation of the mixture and extraction of the distillate with dichloromethane. The separation of the components was done on the SCOT-K20M column, and the identification via mass spectral data and gas chromatographic order of elution.

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Table I. Identification of Components Found in the Basic Fraction

| Peak Number | Name of Compound | Retention on GC Columns | | | Comment[a] |
		SCOT-K20M	CAP-DEGS	M.S.	
3	Pyrazine	+	+	+	P
4	2-Methyl pyrazine	+	+	+	P
5	2,5-Dimethyl pyrazine	+	+	+	P
6	2,6-Dimethyl pyrazine	+	+	+	P
7	2,3-Dimethyl pyrazine	+	+	+	P
8	2-Ethyl-3-methyl pyrazine	+	+	+	P
9	2-Ethyl-3,5-dimethyl pyrazine	+	+	+	P
10	2-Ethyl-3,6-dimethyl pyrazine	+	+	+	P
14	2-Ethyl-3,5,6-trimethyl pyrazine	+	+	+	P
14a	5-Methyl furfural	+	+	+	P
15	Benzaldehyde	+	+	+	P
16	O-Methoxy phenol	+	+	+	P
17	N-Methyl-2-formylpyrrole			?	T
18	2,3,5-Trimethyl pyrazine	+	+	+	T
19	Tetramethyl pyrazine	+	+	+	P
22	p-Cresol	+	+	+	P
23	m-Cresol	+	+	+	P

[a] P = positive identification; T = tentative identification.

RESULTS

The separation of the volatile basic fraction of the hydrolyzed soybean protein on a SCOT Carbowax 20M (0.02 in.) column is shown in Figure 1.

Identification of the compounds was based primarily on mass spectroscopy and gas chromatographic retention factors. The data of the unknowns were compared to authentic compounds. The compounds identified are presented in Table I. The peak numbers refer to those of Figure 1.

A (+) symbol in the gas chromatography column indicates retention time agreement with known compounds. A (+) symbol in the mass spectra column indicates mass spectral agreement with published or known spectra.

DISCUSSION

The major components of the volatile basic fraction are alkylated pyrazines. The pyrazines have been isolated in a number of roasted food products, including peanuts (Mason et al., 1966, 1967), cocoa beans and butter (Rizzi, 1967; van Praag et al., 1968), and coffee (Bondarovich et al., 1967; Goldman et al., 1967; Reichstein and Beitter, 1930; Viani et al., 1965) and potato chips (Deck and Chang, 1965).

Newell et al. (1967) and Dawes and Edwards (1966) suggested that amino acids and carbohydrates are among the precursors of typical nut-like flavor. Experiments with simple aldose-amine-water mixtures (2:1:3 by weight) adjusted to pH 9.0 and refluxed for 2 hr proved that such systems may, indeed, give rise to pyrazines (Dawes and Edwards, 1966).

At the present time there is some speculation as to the mechanism of production. Dawes and Edwards (1966) suggested that the pyruvaldehyde, which is a known reaction product of sugar-amine mixtures (Nodzu, 1935), participates in the Strecker degradation of amino acids. The reaction is due to the reductone nature of the compound, and the products may be either 2-amino-propanal or 1-amino-propanone. Subsequent self-condensation of either of the products and oxidation will produce 2,5-dimethyl pyrazine. Figure 2 outlines the mechanism. If R^1 were CH_3 and R^2 were H, then the above product would be produced. However, if the 2-amino-propanal and 1-amino-propanone were condensed together and oxidized, then 2,6-dimethyl pyrazine would be
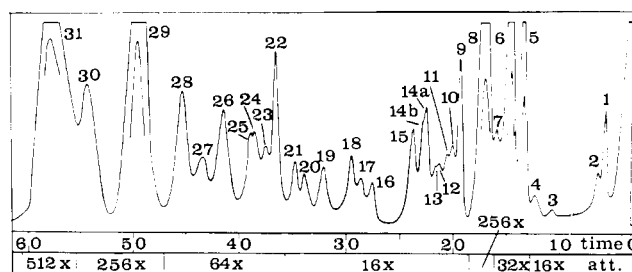

Figure 1. Gas chromatogram of the basic fraction as resolved on a SCOT Carbowax 20M column

the result. If the Strecker degradation takes place with diacetyl (i.e., $R^1 = CH_3$ and $R^2 = CH_3$), a known nonenzymatic browning product (Nodzu, 1935), then self-condensation will produce tetramethyl pyrazine. The condensation of the diacetyl product and 2-amino-propanal or 1-amino-propanone will yield 2,3,6-trimethyl pyrazine and 2,3,4-trimethyl pyrazine, respectively. All these components have been isolated in the hydrolyzate.

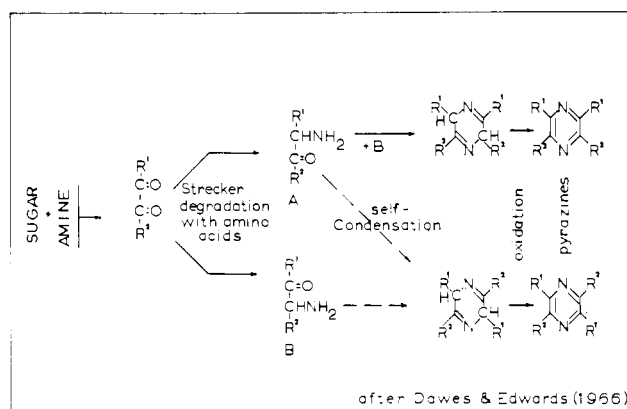Newell et al. (1967) suggested that the mechanism of conversion of amino acids and carbohydrates to volatile pyrazine

Figure 2. Scheme of the production of alkyl substituted pyrazine compounds

compounds is via a different route than detailed above. Their mechanism suggests the production of 1-amino-propane from the decarboxylation of a Schiff base cation. This, of course, will self-condense as shown in the Dawes and Edwards (1966) scheme to 2,5-dimethyl pyrazine. This total mechanism does not, however, predict the production of other alkylated isomers of pyrazine.

As previously noted, the odor of the pyrazine is that of roasted nuts. The odor notes of normal hydrolyzed soy protein do not contain the roasted nut-like odor. However, if one were to increase the pH to the alkaline side, the odor of roasted nuts does occur. This was clearly shown in our laboratory when a solution of hydrolyzed soy protein in the alkaline state was compared to a water solution of 2,5-dimethyl pyrazine. Both samples were characterized by a panel to have a nut-like aroma.

Deck and Chang (1965) established the odor threshold of 2-methyl pyrazine and 2,5-dimethyl pyrazines at 1 ppm. A quantitative study of the pyrazines in hydrolyzates should give some indication as to the importance of the pyrazines to the aroma of the hydrolyzates in the alkaline or, indeed, acid state.

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