

not change, a breakdown and resynthesis of proteins occurs during storage. Breakdown and resynthesis of proteins have already been found by Pech *et al.* (1970) during storage of pears at 4°.

The band marked with an arrow (Figure 2, R_m 0.92) could indicate that the apple has reached the condition of "just acceptable." Further investigation is necessary to characterize this protein and to see whether its appearance can be used as a criterion for fruit quality.

ACKNOWLEDGMENT

I wish to thank Gerrit J. Mooi for his skillful work on the protein patterns, Hendrik Zonneveld for enzymatic analysis, Willem Klop for valuable discussions, Ricardus A. Hilhorst for statistical calculations, David Yarrow (Centraal Bureau voor Schimmelcultures, Delft), Gustaaf A. de Zoeten (University of Wisconsin, Madison, on sabbatical leave at the Department of Virology, Agricultural University, Wageningen) for reading the manuscript, and John Christopher Rigg (Pudoc, Wageningen) for English revision.

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Received for review October 10, 1972. Accepted March 12, 1973.

Identification of Volatile Compounds from Heated L-Cysteine·HCl/D-Glucose

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The volatile compounds produced in a heated L-cysteine·HCl/D-glucose system were collected on Porapak Q and transferred to a capillary column for separation. Identification of compounds was accomplished by tandem gas chromatography-

mass spectrometry. The compounds identified included acyclic α -dicarbonyls, furan derivatives, aromatic and aliphatic monocarbonyls, and sulfur-containing heterocyclics.

In spite of the tremendous amount of work on the chemistry of nonenzymatic browning, relatively little has been reported concerning the volatiles produced from sulfur-containing amino acids in heated food systems. Kobayashi and Fujimaki (1965) found that boiling cysteine with α -diketones produced mercaptoacetaldehyde, hydrogen sulfide, and acetaldehyde and they discussed the route of formation of hydrogen sulfide. The pyrolysis of sulfur-containing amino acids was studied by Fujimaki *et al.* (1969) and these workers reported the formation of eight volatile compounds, including 2-methylthiazolidine, from pyrolyzed cysteine and cystine. The pyridoxal-catalyzed elimination of hydrogen sulfide or methyl mercap-

tan from L-cysteine, S-methyl-L-cysteine, and DL-methionine in the presence and absence of metal ions was quantitatively studied by Gruenwedel and Patnaik (1971).

Herz and Shallenberger (1960) described the aromas produced upon heating various amino acids with glucose. Arroyo and Lillard (1970) conducted odor evaluations and chemical analyses, and studied the effect of pH on the nonenzymatic browning reaction products formed from equimolar concentrations of glucose and the sulfur-containing amino acids. They concluded that none of the three mixtures studied emitted an odor associated with meat. The reaction products from the nonenzymatic browning of glucose and methionine were studied by Lindsay and Lau (1972) and they concluded that methionine was responsible for the boiled potato-like aroma of the reaction mixture. Stoll *et al.* (1967) identified a number of aliphatic and heterocyclic sulfur-containing compounds in

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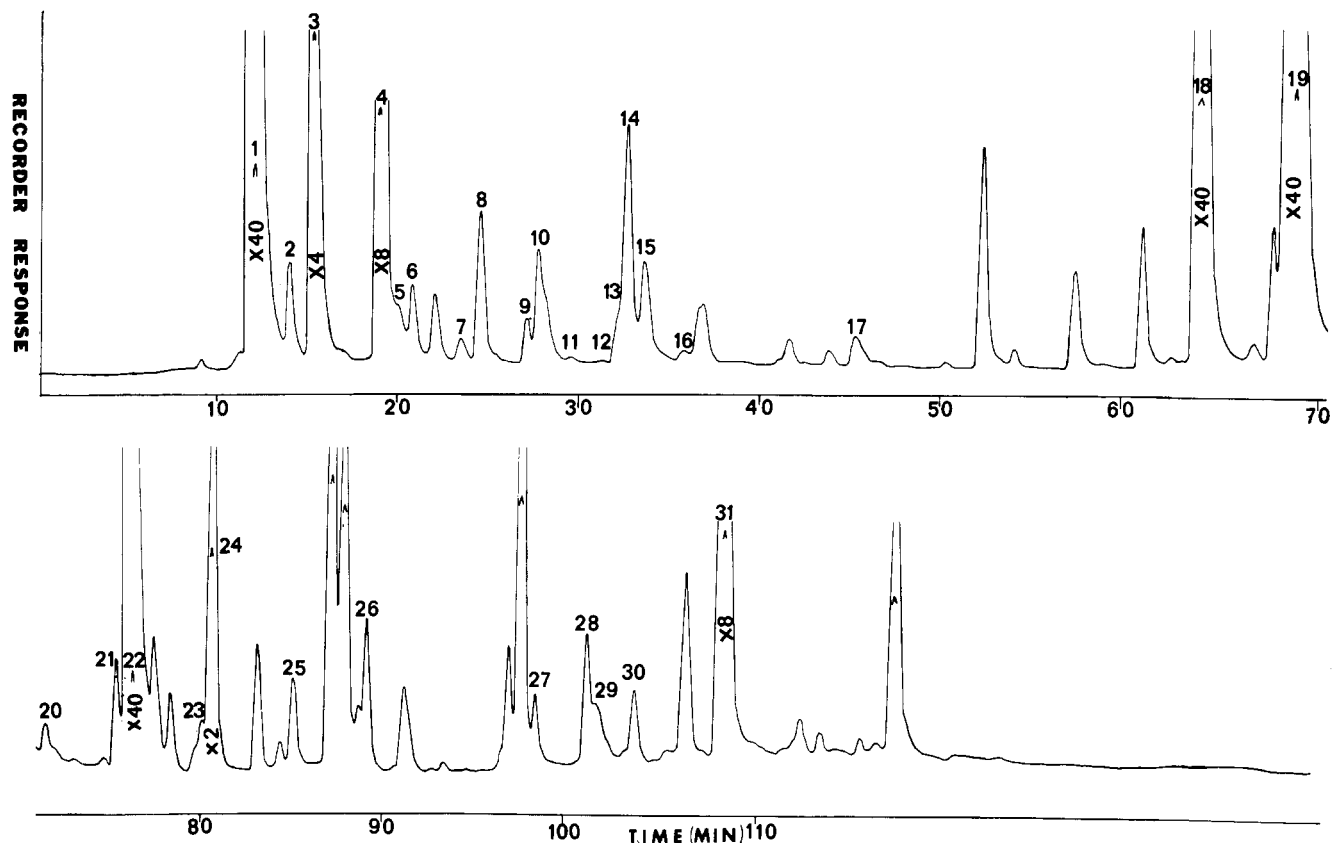


Figure 1. Chromatogram of volatiles from heated L-cysteine·HCl/D-glucose.

coffee aroma, and more recently Mussinan and Katz (1971) and Wilson *et al.* (1972) reported a variety of sulfur-containing compounds in heated meat systems.

Our purpose was to isolate and identify the volatile compounds produced in a heated L-cysteine·HCl/D-glucose system.

EXPERIMENTAL SECTION

Sample Preparation. Twelve grams of L-cysteine·HCl and 24 g of α -D-glucose were dissolved in 12 ml of deionized distilled water at 60°. The mixture was transferred to a three-neck round-bottomed flask (100 ml) and refluxed for 15 min to initiate the reaction. The condenser was then removed and two stainless steel traps (102 mm \times 6 mm i.d.) packed with 100–120 mesh Porapak Q were fitted onto the three-neck reaction flask. The trap, which collected the volatile compounds produced in the reaction flask, was connected to a vacuum pump, while the other trap served as an air filter for the incoming air. The vacuum was adjusted to maintain a flow rate of 30 ml/min throughout the reaction (100° for 50 min).

The Porapak Q traps had been conditioned at 190° for 30 min and then at 100° for 24–48 hr, with a nitrogen gas flow rate of 60 ml/min prior to use. Subsequent gas chromatographic (gc) analysis of conditioned traps revealed no significant background response at the sensitivity used for analysis.

Sample Transfer. The Porapak Q trap was removed from the reaction flask, heated to 50° with a hooded heat gun in which the heating element was controlled by a variable transformer, and the water was removed with a stream of nitrogen (20 ml/min) for 45 min. The trap was then reversed and heated to 165° and the volatiles, eluted with nitrogen (20 ml/min), were condensed in a stainless steel trap (6 in. \times 0.05 in. i.d.) immersed in a slurry of Dry Ice in 2-methoxyethanol. The trap was sealed with plug fittings and held in powdered Dry Ice until it was

connected to a modified inlet system (Scanlan *et al.*, 1968) and the volatiles were flashed at a temperature of 245° into a capillary column (500 ft \times 0.03 in. i.d.) coated with a mixture of Carbowax 20M and Versamid 900 according to the procedure described by Mon (1971).

Gas-Liquid Chromatography-Mass Spectrometry.

The carrier gas flow rate was 12 ml of helium/min at 70°. The flame ionization detector temperature was 210° and column temperature was 70° for 5 min; then it was programmed to 190° at 1.2°/min. The gc was fitted with an effluent splitter which directed 17% of the effluent to the flame ionization detector and the remainder to the mass spectrometer (ms). The heated connection between the gc and the ms was fitted with a micro-volume switching valve (Carle Instruments, Inc., Fullerton, Calif.) and a membrane-type carrier gas separator. The valve allowed diversion of air, which may have inadvertently leaked into the system during sample transfer, from the ion source.

Mass spectra were obtained using an Atlas CH-4 ms. Scans were made from m/e 25 to m/e 250 in 2.5 sec for early gc peaks and in 5.0 sec for later peaks. The ms operating conditions were: filament current, 12 μ A; electron voltage, 70 eV; accelerating voltage, 3.0 kV; analyzer pressure 2×10^{-6} mm; and multiplier voltage, 1.6 kV.

RESULTS AND DISCUSSION

The compounds identified are listed in Table I. The location of each compound is indicated in the chromatogram obtained from the flame ionization detector (Figure 1). Compounds for which gc retention is indicated (+) had relative retention time values within 5% of the values for the corresponding known compound. The absence of a positive sign indicates the known was not available for comparison. All compounds listed in Table I had mass spectral fragmentation patterns which agreed with published spectra.

The aroma from the L-cysteine·HCl/D-glucose system

Table I. Compounds Identified from Heated D-Glucose/L-Cysteine·HCl

Compounds	Peak (Figure 1)	gc retention	ms reference
2-Butanone	1	+	Stoll <i>et al.</i> (1967)
Methyl vinyl ketone	2	+	ASTM (1969)
Benzene	2	+	API (1948)
Diacetyl	3	+	Stoll <i>et al.</i> (1967)
Thiophene	4	+	API (1948)
Crotonaldehyde	5	+	ASTM (1969)
Toluene	5	+	API (1948)
2,3-Pentadione	6	+	Stoll <i>et al.</i> (1967)
Hexanal	7	+	Stoll <i>et al.</i> (1967)
2-Methylthiophene	8	+	API (1948)
3-Methylthiophene	9	+	API (1948)
<i>p</i> -Xylene	10	+	API (1948)
<i>m</i> -Xylene	11	+	API (1948)
2,5-Dimethylthiophene	12	+	API (1948)
2,3-Dimethylthiophene	13	+	API (1948)
2-Ethylthiophene	14	+	API (1948)
Cyclopentanone	15	+	Stoll <i>et al.</i> (1967)
Crotyl alcohol	16	+	ASTM (1969)
Cyclohexanone	17	+	API (1948)
Furfural	18	+	Stoll <i>et al.</i> (1967)
2-Acetylfuran	19	+	Stoll <i>et al.</i> (1967)
Benzaldehyde	20	+	Stoll <i>et al.</i> (1967)
Dihydro-3(2H)-thiophenone	21	+	Stoll <i>et al.</i> (1967)
5-Methyl-2-furfural	22	+	Stoll <i>et al.</i> (1967)
2-Thiophenethiol	23	+	API (1948)
2-Acetyl-5-methylfuran	24	+	Stoll <i>et al.</i> (1967)
3-(Methylthio)thiophene	25	+	API (1948)
2-Formylthiophene	26	+	Stoll <i>et al.</i> (1967)
2-Acetylthiophene	27	+	Stoll <i>et al.</i> (1967)
5-Methyl-2-formylthiophene	28	+	Stoll <i>et al.</i> (1967)
2,3-Benzothiophene	29	+	Stoll <i>et al.</i> (1967)
2-Propionylthiophene	30	+	Stoll <i>et al.</i> (1967)
Thieno[3,2-b]thiophene	31	+	Stoll <i>et al.</i> (1967)

was described as stewed chicken at the earlier stages of heating. In several preliminary experiments in which the system was buffered at higher pH values, caramel aromas were observed. We left the system unbuffered because we felt the stewed chicken aroma would be the more interesting one to study. The pH of the reaction mixture changed from 1.7 to 1.6 during the heating period.

Diacetyl and 2,3-pentanedione are examples of acyclic α -dicarbonyl compounds derived by dehydration and fission of the sugar radical (Hodge *et al.*, 1972). Several furan derivatives which are characteristic of nonenzymatic browning systems were identified. The complete lack of nitrogen heterocyclics was surprising, since alkylpyrazines have been reported in a variety of browning systems (Hodge *et al.*, 1972). Perhaps either the relatively low pH, the high water activity, and/or the relatively low temperature prevented pyrazine formation.

The relatively large number of sulfur heterocyclics was interesting, if not unexpected. Many of these compounds have been identified in the aroma of coffee (Stoll *et al.*, 1967), and the occurrence of sulfur heterocyclics in the volatiles from model meat systems has been reported (Mussinan and Katz, 1971; Wilson *et al.*, 1972). The contribution of the sulfur heterocyclics to aroma and the elucidation of their mechanism of formation awaits further investigation.

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Received for review November 20, 1972. Accepted March 8, 1973. The authors thank the Nutrition Foundation, Inc., for financial support of this investigation. Technical Paper No. 3476, Oregon Agricultural Experiment Station, Oregon State University, Corvallis, Oregon.