

Acknowledgments

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Central Food Technological Research Institute
Mysore, 2
India

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References

- ¹ Sastry, L. V. L., Bhatia, B. S., & Lal, Girdhari, *J. Fd Sci.*, in the press
² Bedrosian, K., Nelson, A. I., & Steinberg, M. P., *Food Tech.*, 1959, **13**, 722
³ Joslyn, M. A., *Food Res.*, 1957, **22**, 1; Siddappa, G. S., Bhatia, B. S., & Lal, Girdhari, *Indian J. appl. Chem.*, 1959, **22**, 199

PRODUCTION OF VOLATILE COMPOUNDS RELATED TO THE FLAVOUR OF FOODS FROM THE STRECKER DEGRADATION OF DL-METHIONINE

By P. E. BALLANCE

A qualitative investigation of the volatile compounds formed during the Strecker degradation of DL-methionine has been carried out. The volatile products were condensed in cold traps and analysed by gas-liquid chromatography. The condensate was shown to consist chiefly of methyl mercaptan, together with traces of other compounds including acrolein and dimethyl sulphide. The suggestion that degradation of methionine first gives rise to methional is supported by the identification of this compound and the demonstration of its decomposition with ninhydrin to methyl mercaptan and acrolein. These findings are discussed in relation to the importance of methionine as a source of flavour in food.

Introduction

The importance of the Strecker degradation¹ of amino-acids in the development of flavour in food has recently been discussed by Keeney & Day.² These workers reported a 'broth- or cheese-like' smell resulting from the reaction of methionine with ninhydrin (indanetrione hydrate). The development of the broth-like odour would be consistent with the formation of methional (3-methylthiopropionaldehyde), the strong odour of which has been recently affirmed by several workers.³⁻⁵

The suggestion that methional is formed by the breakdown of methionine is supported by the experimental work of Hunter & Potter⁶ who obtained 100% yields of methional from methionine treated with ninhydrin. Bayer⁷ has claimed, without giving any experimental details, that propionaldehyde is the major volatile product of the hypochlorite breakdown of methionine. In view of these conflicting reports a detailed qualitative examination of the volatile products of methionine degradation was undertaken.

Experimental

Physical trapping of volatile compounds

DL-Methionine (40 mg., California Foundation for Biochemical Research, Los Angeles, California, U.S.A.) was warmed (80°) with ninhydrin (200 mg.), water (10 ml.), sodium chloride (2 g.) and sodium dihydrogen phosphate (1 g.), for 2 h. A stream of nitrogen was drawn through the reactants at slightly reduced pressure (200 mm.); the emergent gas stream was led through two traps cooled in solid carbon dioxide, into a U-tube (trap A) cooled with liquid nitrogen.

An alternative method involved the removal of water from the emergent nitrogen stream by passing it through warmed (80°) calcium chloride, and trapping the volatile compounds in a Y-shaped trap (B) cooled in liquid nitrogen. Trap B was constructed of tubing with 4 mm. i.d. with the short 14-cm. limb entering the long (17-cm.) limb 3 cm. from the base: condensed vapour collected in the base of the long limb.

Chemical trapping of volatile compounds

To obtain further information concerning the identity of the volatile compounds the emergent nitrogen stream was bubbled through particular reagents. A white precipitate is formed if dimethyl sulphide or dimethyl disulphide is present in a gas stream bubbled through mercuric chloride (3% w/v) solution: a precipitate is also formed if methyl mercaptan is present, but this compound can be removed by passage of the gas stream through mercuric cyanide solution (4% w/v). The details of these methods of trapping volatile sulphur compounds have been described by Challenger & Charlton,⁸ and Challenger.⁹

Aldehydes or ketones react with 2,4-dinitrophenylhydrazine reagent (0.2% w/v solution in 2N-HCl) to form 2,4-dinitrophenylhydrazones, which are identified either by determination of melting point or by regeneration of the carbonyl compound with levulinic reagent, followed by gas-liquid chromatography.

Regeneration of carbonyl compounds

The 2,4-dinitrophenylhydrazones (100 mg.) were treated with levulinic reagent (1 vol. levulinic acid: 9 vol. N-H₂SO₄) according to the method of Keeney.¹⁰ The carbonyl compounds evolved were condensed at liquid-nitrogen temperature in trap B. The condensate was dissolved in 1 ml. of ether, and 0.025 μ l. samples of the ether solution removed by micropipette for analysis by gas-liquid chromatography.

Following levulinic regeneration the more volatile carbonyl compounds were collected in trap A (by the method previously described). The U-tube was warmed to room temperature and 0.5-ml. vapour samples removed for gas-liquid chromatographic analysis.

Gas-liquid chromatography

The Pye Argon Chromatograph was used for gas-liquid chromatographic analyses. All columns (glass) were 4 ft. long and 4–5 mm. i.d., and were packed with Celite (Gas Chromatography Ltd., G-cel 100–120 mesh) impregnated with stationary liquid. For high-temperature analyses (100°) the stationary liquid was 5% polypropylene adipate (Briggs & Townsend Ltd.), for low-temperature analyses (22°) 5% dinonyl phthalate (May & Baker Ltd.) or 10% polyethylene glycol 400 (L. Light & Co. Ltd.) were used. The stationary liquids were dissolved in a volatile solvent, mixed with Celite and the solvent was evaporated while stirring. The resulting powder was introduced into the columns and packed down by vigorous tapping.

For low-temperature separation 0.5-ml. vapour samples were introduced by means of an Agla syringe equipped with a fine hypodermic needle. The needle was inserted into the rubber tubing conveying argon to the top of the column. Liquid samples for the high-temperature column were placed directly into the top of the column by micropipette. For all analyses the argon flow rate was 40–45 ml./min.

All qualitative results are presented as relative retention volumes. Values obtained with low-temperature columns are related to isobutyraldehyde; methyl n-heptanoate (prepared by diazomethane treatment of n-heptanoic acid) was selected as internal standard for the polypropylene adipate column. Methyl mercaptan was obtained from Eastman Organic

Chemicals, Rochester 3, New York, U.S.A. : pure methional was prepared by the method of Pierson *et al.*¹¹

Results

Degradation of DL-methionine

The volatile compounds collected in trap A from a warmed mixture of methionine with ninhydrin in the presence of water, sodium chloride and sodium dihydrogen phosphate, were analysed by gas-liquid chromatography on the low-temperature columns. On both dinonyl phthalate and polyethylene glycol columns one large constituent was found, the relative retention volume of which corresponded with that of methyl mercaptan (Table I). Trace amounts of other volatile compounds, acrolein, isobutyraldehyde, dimethyl sulphide and dimethyl disulphide were identified by comparison with known compounds. The finding of a compound having the same relative retention volume as isobutyraldehyde might well be due to a trace of valine as an impurity in the methionine. An unknown compound was also detected. It was possible to confirm the identification of methyl mercaptan, a white precipitate being formed when the emergent gas stream was led through a mercuric cyanide solution. The precipitate was boiled with a quantity of water to remove any combined mercuric cyanide,^{8, 9} dried in a desiccator : it melted with decomposition at 174° (m.p. of pure mercury dimethyl mercaptide, 174-175°⁸).

Table I

Relative retention volumes (R.R.V.s) of the volatile products of the Strecker degradation of methionine

Internal standard isobutyraldehyde = 1

Unknown volatile compounds				Known compounds		
Peak position	Peak size	No. of observations	Mean R.R.V.	Suspected compound	No. of observations	Mean R.R.V.
<i>Dinonyl phthalate column</i>						
1	v. large	15	0.17	Methyl mercaptan	7	0.18
2	trace	4	0.47	Dimethyl sulphide	8	0.48
3	trace	13	0.58	Acrolein	5	0.58
4	trace	10	1.00	Isobutyraldehyde	18	1.00
				Dimethyl disulphide	1	7.29
<i>Polyethylene glycol column</i>						
1	v. large	11	0.29	Methyl mercaptan	4	0.28
2	trace	8	1.40	Acrolein	2	1.42
3	trace	7	1.05	Isobutyraldehyde	17	1.00
4	small	4	5.89	Unknown	—	—
5	small	2	7.53	Dimethyl disulphide	1	7.18

To explain the preponderance of methyl mercaptan it was assumed that the initial step in the reaction sequence was the formation of methional, which subsequently gave rise to acrolein and methyl mercaptan : the tendency of acrolein to polymerise would account for its being found only in trace amounts. In an attempt to establish the presence of methional the degradation of methionine was repeated and the emergent gas stream passed through 2,4-dinitrophenylhydrazine solution. A heavy yellow precipitate of a 2,4-dinitrophenylhydrazone formed, was filtered off, recrystallised and its melting point (and a mixed melting point) found to correspond with the value 123° obtained with crystals of the 2,4-dinitrophenylhydrazone of pure methional. As a further check the hydrazone was treated with levulinic reagent and the volatile compounds produced were dried (CaCl₂) and condensed in trap B. The condensate was chromatographed at 100° on the polypropylene adipate column and a component was detected having the same relative retention volume (2.09) as a sample of pure methional.

The breakdown of methional

As the presence of methional had been established, the possibility that it decomposes under certain conditions was investigated. Methional, prepared from acrolein and methyl

mercaptan,¹¹ was twice distilled and its purity determined by gas-liquid chromatography on polyethylene glycol. Nitrogen was swept (2 h.) through the methional and the condensate obtained by passage of the emergent gas through a U-tube cooled in liquid nitrogen was shown to consist of very slight traces (peak heights 3-4 mm.) of acrolein and methyl mercaptan. Heating the methional (0.4 ml.) in the presence of water (10 ml.) did not bring about any significant decomposition; acrolein and methyl mercaptan peaks (heights 1.1-1.2 cm.) were revealed with the polyethylene glycol column.

Methional (0.4 ml.) was heated (2 h.) with 1.124 g. of dry ninhydrin, a nitrogen stream was swept through the apparatus and volatile matter collected in trap A. Gas-liquid chromatography on both polyethylene glycol and dinonyl phthalate columns (Table II) showed the presence of methyl mercaptan, dimethyl sulphide and acrolein (the peak heights were greater than 10 cm.). These volatile reaction products were also identified chemically; the emergent gas stream, when passed through a train of three pairs of reagent solutions, produced a white precipitate (methyl mercaptan) in mercuric cyanide solution, an orange precipitate (acrolein) in 2,4-dinitrophenylhydrazine reagent, and a white precipitate due to dimethyl sulphide in mercuric chloride solution. The latter precipitate was warmed with 5N-NaOH and the volatile material bubbled through a solution of mercuric cyanide, in which no precipitate formed, and then through mercuric chloride solution where the presence of dimethyl sulphide was revealed by the formation of a white precipitate (Challenger & Charlton⁶). Treatment of the orange 2,4-dinitrophenylhydrazone with levulinic reagent and chromatography of the volatile material on polyethylene glycol established the identity of acrolein (relative retention volume 1.41).

Table II

Identification of the volatile products from the reaction of methional and dry ninhydrin

Relative retention volumes (R.R.V.s) based on isobutyraldehyde = 1				
Column	Peak position	R.R.V.	Suspected compound	R.R.V. of suspected compound
Polyethylene glycol 400, 10%	1	0.26	Methyl mercaptan	0.26
	2	0.47	Dimethyl sulphide	0.48
	3	1.40	Acrolein	1.40
Dinonyl phthalate, 5%	1	0.16	Methyl mercaptan	0.18
	2	0.47	Dimethyl sulphide	0.48
	3	0.61	Acrolein	0.61

The similarity of the volatile products of methional breakdown (Table II) and the compounds produced by ninhydrin treatment of methionine (Table I) leaves little doubt that methional is the first recognisable product of the Strecker degradation of methionine, in agreement with the work of Hunter & Potter.⁶

Although Bayer⁷ reported the evolution of propionaldehyde following hypochlorite breakdown of methionine, at no time under the conditions used in the present work was propionaldehyde found in any of the volatile mixtures. Even heating methionine with sodium hypochlorite solution, according to the method of Langheld,¹² gave rise to no identifiable quantities of volatile materials. Using the same conditions, however, copious amounts of acetaldehyde were produced from alanine.

The finding that the Strecker degradation of methionine gave rise to methyl mercaptan as the major volatile product is consistent with reports that methyl mercaptan is produced during breakdown of methionine by micro-organisms.¹³

Discussion

The present investigation has emphasised the importance of methionine as a source of volatile sulphur compounds, all of which have been associated with the flavours of foodstuffs. Methional, the first volatile product of methionine degradation, has been associated with the 'sunlight' flavour of milk.¹⁴ Although Day *et al.*⁴ have examined the rôle of methional as a flavour compound it appears capable of further breakdown to the more strongly odorous¹⁵ methyl mercaptan. By the use of gas-liquid chromatography and mass spectrometry this

mercaptan has been identified as a constituent of meat flavour by Stahl¹⁶ and Merritt *et al.*¹⁷ The latter workers also detected dimethyl sulphide in meat odour, whereas previously it had been found only in the volatile compounds from irradiated milk¹⁸ and meat.¹⁶ Dimethyl disulphide, formed by the oxidation of traces of methyl mercaptan, has been shown to be a constituent of cooked cabbage flavour;¹⁹ in the cabbage, however, the presence of dimethyl disulphide has been attributed to the decomposition of S-methyl-L-cysteine sulphoxide, which would almost certainly yield methyl mercaptan as a primary product.^{9, 20}

Although the experimental conditions employed in the present work differed considerably from those discussed by Challenger⁹ when considering the origin of dimethyl sulphide from a thetin-like structure, no other explanation can be suggested to account for the production of dimethyl sulphide.

It is clear that the Strecker degradation of methionine might well, to some extent, be responsible for the volatile sulphur compounds found in the odours from non-irradiated foods.

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Research Dept.
Bovril Limited
London, E.C.1

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References

- ¹ Schönberg, A., & Moubacher, R., *Chem. Rev.*, 1952, **50**, 261
- ² Keeney, M., & Day, E. A., *J. Dairy Sci.*, 1957, **40**, 874
- ³ Patton, S., & Barnes, I., *Food Res.*, 1958, **23**, 221
- ⁴ Day, E. A., Keeney, M., & Stahl, W., *Food Res.*, 1958, **23**, 130
- ⁵ Oro, J. F., Guidry, C. L., & Zlatkis, A., *Food Res.*, 1959, **24**, 240
- ⁶ Hunter, I. R., & Potter, E. F., *Analyt. Chem.*, 1958, **30**, 293
- ⁷ Bayer, E., 'Gas Chromatography', ed. Desty, D. H., 1958, p. 333 (London: Butterworths)
- ⁸ Challenger, F., & Charlton, P. T., *J. chem. Soc.*, 1947, p. 424
- ⁹ Challenger, F., 'Aspects of the Organic Chemistry of Sulphur', 1959, pp. 16-19, 37, 40, 50 (London: Butterworths)
- ¹⁰ Keeney, M., *Analyt. Chem.*, 1957, **29**, 1489
- ¹¹ Pierson, E., Giella, M., & Tishler, M., *J. Amer. chem. Soc.*, 1948, **70**, 1450
- ¹² Langheld, K., *Ber. dtsh. chem. Ges.*, 1909, **42**, 392, 2360
- ¹³ Segal, W., & Starkey, R. L., *Analyt. Chem.*, 1953, **25**, 1645; Miwatani, T., Omukai, Y., & Nakada, D., *Med. J. Osaka Univ.*, 1954, **5**, 347
- ¹⁴ Patton, S., *J. Dairy Sci.*, 1954, **37**, 446
- ¹⁵ Patton, S., & Josephson, D. V., *Food Res.*, 1957, **22**, 316
- ¹⁶ Stahl, W. H., 'Chemistry of Natural Food Flavours', eds. Mitchell, J. H., jun., *et al.*, 1957, p. 58 (Washington, D.C., U.S.A.: Dept. of the Army Research & Development Command, Quartermaster Food & Container Inst. for the Armed Forces)
- ¹⁷ Merritt, C., jun., Bresnick, S. R., Bazinet, M. L., Walsh, J. T., & Angelini, P., *J. agric. Fd Chem.*, 1959, **7**, 784
- ¹⁸ Day, E. A., Forss, D. A., & Patton, S., *J. Dairy Sci.*, 1957, **40**, 932
- ¹⁹ Dateo, G. P., Clapp, R. C., MacKay, D. A. M., Hewitt, E. J., & Hasselstrom, T., *Food Res.*, 1957, **22**, 440
- ²⁰ Synge, R. L. M., & Wood, J. C., *Biochem. J.*, 1956, **64**, 252