Origin of Methanol and Dimethyl Sulphide from Cooked Foods

A SURVEY of the volatiles produced from a range of cooked vegetables showed that both methanol and dimethyl sulphide were usually present as major components[1]. When a commercial sample of instant coffee powder was examined, however, no methanol and only small amounts of dimethyl sulphide were detected, although other common volatiles of low boiling point which are engendered from amino-acids[2] were conspicuously present. It appeared likely, therefore, that both methanol and dimethyl sulphide were produced from vegetables on cooking by some system which was not present in the coffee powder.

One obvious possible origin of methanol is pectin, which was suggested as the source in tea[3], although in this case it was believed to be produced by prior enzyme action (cf. ref. 4). We have now shown that when purified pectin preparations are boiled in phosphate buffer (5 mg/ml. 0·1 M, *p*H 6·5), they hydrolyse to yield methanol in comparable amounts as are formed from cooked vegetables. Methyl esters of simple carboxylic acids are not hydrolysed to any detectable extent under these conditions, and it appears probable that **most of the methanol observed from cooked foods arises from the non-enzymatic hydrolysis of pectin.**

Although dimethyl sulphide is known to be produced by heating a solution of methionine[2,5] either alone or in presence of sugars and other natural oxidants, the amounts formed are relatively small[2]. Obata and Mizutana[5], however, reported that heating methionine in the presence of plant material known to contain pectin increased the yield of the sulphide. We have now shown that heating methionine and purified pectin at 100° in buffer solutions (*p*H 6·5) at concentrations of 1–5 mg/ml. produces quantities of dimethyl sulphide together with dimethyl disulphide, methane thiol, acrolein and methanol, in the same order as obtained from certain cooked vegetables. It appeared likely that dimethyl sulphide is produced by a mechanism similar to that suggested by Lavine *et al.*[6] for the reaction of methionine with methanol in the presence of strong acids. However, the use of methanol alone, methanol and galacturonic acid, or methyl esters of simple carboxylic acids instead of pectin failed to increase the amount of dimethyl sulphide produced over that obtained from methionine alone. Methyl donors, such as choline and betaine, only showed trace activity. That pectin was acting as a methyl donor was shown by an examination of the decomposition of methionine. When heated with ninhydrin[2,7] this compound gives mainly ethane thiol, but when pectin is present ethyl methyl sulphide is also produced. The influence of *p*H and other metabolites on the transmethylation reaction has still to be examined. It appears likely, however, that although dimethyl sulphide may be produced enzymatically[4] or from other sources[8], part at least comes from the reaction described, and this may be of importance in enhancing the flavour of manufactured foods.

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Self, R., Casey, J. C., and Swain, T., *Chem. and Indust.*, 863 (1963).
Casey, J. C., Self, R., and Swain, T., *Proc. First Intern. Congr. Food Sci. Technol.* (London, 1962).
Ramaswamy, M. S., and Lamb, J., *J. Sci. Food Agric.*, **9**, 46 (1958).
Niegisch, W. D., and Stahl, W. H., *Food Res.*, **21**, 657 (1956).
Obata, Y., and Mizutana, J., *Bull. Agric. Chem. Soc., Japan*, **25**, 36 (1961).
Lavine, T. F., Floyd, N. F., and Cammaroti, M. S., *J. Biol. Chem.*, **207**, 107 (1953).
Hunter, I. R., and Potter, E. F., *Anal. Chem.*, **30**, 293 (1958).
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Independence of the Formation of Extracellular Protease on the Amino-acid Level in the Cells of *Bacillus megatherium*

SYNTHESIS of extracellular protease in micro-organisms is repressed in the presence of free amino-acids in the medium[1–3]. However, the formation of intracellular enzyme is not influenced by amino-acids. The quantitative relationship between the amount of amino-acids present in the medium, their level in the cells and inhibition of enzyme formation was investigated during short-time incubation.

The culture grown up overnight on a solid synthetic *C/G*[4] medium was intensively aerated for 2 h in the same liquid medium at 35° C. The cells (0·75 mg dry wt./ml.) were washed and incubated at 35° C in the *C/G* medium containing different amounts of casamino-acids and calcium chloride (1×10^{-3} M) for the stabilization of the enzyme. After incubation for 1 h on a shaker (100 strokes/min, amplitude 6 cm) 100 μg/ml. of chloramphenicol was added. The proteolytic activity was determined in supernatant after centrifugation of the cells. No accumulation of the enzyme occurred in the cells during incubation with amino-acids. The free amino-acids were extracted from the washed cells by boiling for 20 min in the distilled water[6] and colorimetric determinations were carried out[7] (Fig. 1). In some experiments the centrifuged cells were not washed before boiling but the found amino-acid levels were almost identical.

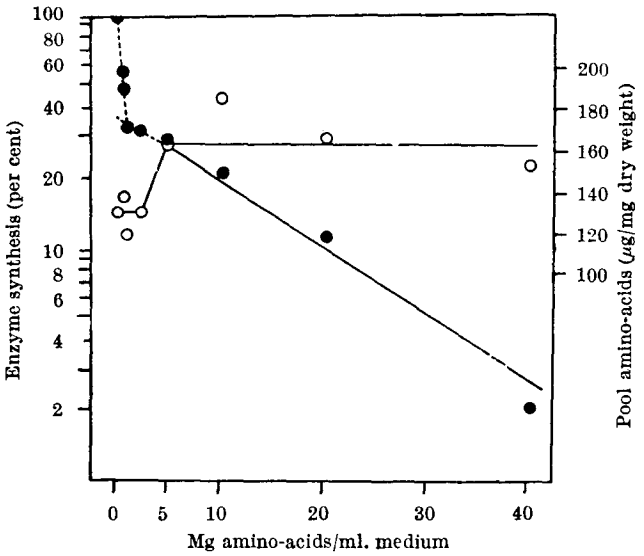

Fig. 1. Relationship between the content of amino-acids in the pool and protease repression. Growing culture of *B. megatherium* was incubated for 1 h on a shaker at 35° in synthetic medium (ref. 4) with Ca^{++} and the aforementioned concentration of casamino-acids. The formation of enzyme was stopped by addition of chloramphenicol

Chloramphenicol inhibited enzyme formation by 95–100 per cent; actinomycine *D* (2·5 μg/ml.) by 90–95 per cent. This shows that, under the conditions of these experiments, the enzyme was synthesized and not only released from the cells. The enzyme formation decreased logarithmically with the increasing amino-acid concentration in the medium. When the concentration of amino-acids was lower than 5 mg/ml., the repression approximated to 60 per cent. During incubation of the cells in the medium containing less than 1 mg casamino-acids/ml. the amino-acids were mostly consumed. The shape of the curve is, therefore, probably deformed in this region.

As is evident from the curve, there are two factors acting in the repression of protease formation by amino-acids, the nature of which is still unknown.

When the cells were incubated with 40 mg/ml. casamino-acids, enzyme formation was decreased by 98 per cent, but the amino-acid level in pool increased by 40 per cent only. When incubated with amino-acid concentration