## Trimethylamine Formation in Relation to the Viable Bacterial Population of Spoiling Fish Muscle

In view of the recent communication in NATURE on this subject<sup>1</sup>, the following comments may be of interest.

Experiments with haddocks stowed in ice<sup>2</sup> have shown that there is no close correlation between the viable count and the trimethylamine content of the flesh. Indeed, the content of dimethylamine, also produced from trimethylamine oxide, follows the general curve of bacterial growth much more closely<sup>2</sup>. Whilst it is most probably true that only certain organisms are capable of reducing the oxide, the empirical fact remains that in haddocks stowed in ice-the usual industrial preservative-the increase in content of trimethylamine has always been found to follow the organoleptic phases of spoilage in a sufficiently consistent way to render the amount of this substance present a useful index of the extent of spoilage. It would seem that, as Dr. Tarr suggests may be the case, the proportion of trimethylamineforming to non-trimethylamine-forming bacteria is fairly constant, at least under the conditions of stowage mentioned above. The content of dimethylamine has similarly been found to be a useful index of spoilage. Indeed, reference to these two indexes together serves satisfactorily to characterize the condition of haddocks at any stage right up to the point of definite staleness.

Finally, whilst it is reasonable to assume, until the contrary is proved, that the flora of the external surfaces of all sea fish is much the same, it has been found that spoilage may pursue different courses in different species. Thus in the dogfish the contents of diamine and triamine never rise during stowage in ice to the extent that might be expected from the much larger amount of oxide present as compared with the haddock. Indeed, diamine is present only in traces during stowage for thirty days; whilst the amount of triamine is only of the same order as that found under similar conditions in the haddock. The explanation of these results may be that the large amount of ammonia formed from urea, present in much larger amounts in the dogfish than in the haddock, causes the trimethylamine oxide reducers to be overgrown by other bacteria. Certainly it has been noticed that after stowage the flora of the dogfish is considerably restricted in types as compared, with that of the haddock.

In this connexion, it is of interest that Dr. Tarr's work, as reported<sup>1</sup>, was done with smoked fish, since it is probable that the constituents of smoke effect considerable alteration in the original flora of the fish. JAMES M. SHEWAN.

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<sup>1</sup> Tarr, H. L. A., NATURE, **142**, 1078 (1938). <sup>2</sup> Shewan, J. M., Report of the Food Investigation Board (London), Sec. 4, p. 75 (1937).

## Determination of Uronic Groups in Polysaccharides

URONIC groupings occurring in polysaccharides are usually determined by measuring the evolution of carbon dioxide on heating with 12 per cent hydro-chloric acid. Campbell, Hirst and Young<sup>1</sup> have pointed out that a small amount of carbon dioxide is liberated under these conditions from a number of pure sugars and polysaccharides in which the presence of uronic groups is not presumed, though not necessarily disproved. The evolution of carbon dioxide from certain acids that might be formed by

the degradation of polyuronides was reported by Norman and Martin<sup>2</sup>. In an investigation of plant celluloses carried out two to three years ago at the Rothamsted Experimental Station, it was sought to establish whether the carboxyl groups demonstrably present were of the uronic type. Yields of carbon dioxide of the order of 0.2-0.3 per cent were considered to be unreliable for the reason just given, and accordingly recourse was had to the determination of the rate of evolution of carbon dioxide from such substances under standard conditions in the hope that this might be employed for demonstrating the presence or absence of uronic groups. No absolute value is involved inasmuch as it is the rate of absorption that is measured, and this is in part a characteristic of the size of apparatus in relation to the sample, the air stream, and absorbing device employed. The oil bath temperature was maintained at 140°, and a constant rate of aeration secured. The distillation was not carried out in an atmosphere of nitrogen; nevertheless the total yields of carbon dioxide from pure substances were not higher than those given by Campbell, Hirst and Young<sup>1</sup>.

Heating was ordinarily continued for five hours, and the absorbing vessels changed every ten minutes in the first hour, every fifteen minutes in the second hour, and thereafter at thirty-minute intervals.

The rate of evolution of carbon dioxide from substances predominantly of a polyuronide nature, such as pectin, was found to be characteristic. The curve for mgm. carbon dioxide evolved per minute per gram exhibited a sharp peak between 20 and 30 minutes from time of reaching the boil. By the end of the first hour, slightly less than 50 per cent of the total carbon dioxide yield was collected. The only sugars exhibiting a sharp peak in carbon dioxide evolution were fructose and sucrose, and in these the maximum occurred earlier, between 10 and 20 minutes from time of boiling. No such sharp maximum was obtained with glucose, from which carbon dioxide was liberated only slowly. Evolution was higher in the second hour of boiling than the first, and the peak, which was ill-defined, appeared to occur at about 75 minutes. By the end of the first hour only about 15 per cent of the total carbon dioxide yield was obtained. The total yield was, in fact, difficult to establish since small quantities continued to be evolved for many hours.

The curve obtained for rice starch was of the same type as that for glucose, but that for cotton cellulose exhibited a peak in the neighbourhood of 30 minutes followed by a steady but not abrupt fall. This was more marked in a sample of Canadian spruce cellulose (not dried), which showed a second but smaller maximum at about 75 minutes. The nature of the curves was such as to suggest that they combined the effect of the uronic grouping in giving an early peak, and that of the hexose material in providing a longer and more steady evolution of carbon dioxide. Such a procedure may therefore be helpful in pointing to the probable presence of uronic groups in polysaccharides, when small quantities only are present. In addition, the total carbon dioxide evolution from a number of plant celluloses was determined.

PERCENTAGE CARBON DIOXIDE EVOLVED FROM CERTAIN CARBO-HYDRATES.

	Celluloses from :					
Glucose			0.19	Wheat straw		0.30
Fructose			0.59	Rye grass		0.31
Sucrose			0.24	Oak		0.54
Rice starch			0.20	Canadian sprud	0.41	
Potato sta	rch		0.20	Jute		0.78
Cotton oxycellulose			1.29	Hemp		0.20
Esparto cellulose			0.16	Manilla hemp		0.65