DETERMINATION OF TOTAL VOLATILE NITROGEN IN CURED FISH PRODUCTS

By V. Krishna Pillai and M. Rajendranathan Nayar
(Central Marine Fisheries Research Station, Mandapam Camp)

Introduction

Estimation of total volatile nitrogenous bases has been generally made use of in routine analysis for the chemical assessment of the degree of spoilage in fish samples. The measurement of this index of spoilage furnishes a reasonably accurate and rapid method for the determination of the keeping quality of cured fish products. In fact it has been pointed out by Velankar (1952) that total volatile nitrogen gives a better index of spoilage than the trimethylamine content. Tarr and Ney (1949) also observed that the test for the amount of trimethylamine present is not likely to prove a very sensitive measurement of the bacterial spoilage of varieties of Pacific coast fishes. It is suggested that trimethylamine is a product during the early stages of spoilage (Collins, 1938; Hess, 1941) and that it may be lost indiscriminately during storage.

The method of estimation of total volatile nitrogen adopted for our experimental investigations is distillation, as suggested by Beatty and Gibbons (1937). The microdiffusion method of Conway and Byrene (1933) can also be used for the same purpose. But when the amount of T.V.N. is greater, as it is likely to be in the case of cured fish, it is generally regarded as safer to employ the distillation method.

The success of this method of estimation depends on the recovery of the volatile bases from fish-muscle extract by steam-distilling with an alkali without causing decomposition of the protein in the muscle. Several conditions have been stipulated for this purpose, *viz.*, use of a mild alkali like sodium borate, a minimum period of distillation, etc. Recently Venkataraman and Chari (1950) made a comparative study of the analytical methods employed in the estimation of total volatile bases and trimethylamine and they suggested that these two constituents can preferably be estimated by steam-distilling a suitable aliquot of the 96% ethyl alcohol extract of the fish sample under reduced pressure.

In the present communication a few similar aspects of the methods of estimation of total volatile nitrogen have been considered. An attempt is made to experimentally determine the comparative efficiency of 96% ethyl alcohol and aqueous trichloracetic acid in extracting the volatile bases from cured fish samples and of the three alkalies, *viz.*, sodium hydroxide, potassium carbonate and sodium borate, in distilling the volatile bases from the extracts.

EXPERIMENTAL

The samples analysed are those experimentally cured in the laboratory as well as those collected from places like Rameswaram, Kundukal Point, Kilakkara, etc. A known weight of the thoroughly ground muscle of the cured fish is allowed to remain in contact with the solvent for a sufficiently long time (15 to 20 minutes) to ensure the complete extraction of the bases. It is then pressed out from the solution and repeatedly washed with the solvent. The extract and the washings are filtered and made up with the solvent to a definite volume. An aliquot portion of the made-up extract is introduced into the microkjeldahl distillation apparatus and distilled under partial vacuum in presence of enough alkali in a current of steam, the distilling vapours being absorbed in 2% boric acid solution (Conway and O'Malley, 1942) containing a drop of Tashiro's methyl red-methylene blue indicator. The distillate is titrated with standard N/50 sulphuric acid.

For comparison, similar estimations were carried out on aqueous extracts of muscle samples prepared by grinding them to a very fine consistency with water and using directly for distillation.

Minimum time of distillation.—It has been observed that an aqueous extract from a definite weight of the sample of cured fish (usually 0·1 g. to 0·3 g.) on being subjected to distillation as described above, gives increasing values of total volatile nitrogen with increase in the duration of the distillation period. From a series of experiments it is concluded that under uniform conditions of distillation an aqueous extract containing 0·1 g. of the fish muscle may be given a minimum time of 4 minutes for the complete evolution of total volatile bases. The relation between the evolution of T.V.N. and the time of distillation in the case of aqueous extracts containing different amounts of the fish muscle has been represented graphically (Fig. 1). It may be inferred from the graph that for extracts containing more than 0·1 g. of the muscle the duration of distillation has to be increased proportionately, but even then, as pointed out by Beatty and Gibbons (1937), complete recovery of all volatile bases has not been possible in the usual practice.

Use of saturated potassium carbonate solution in distillation.—The alkali used in our earlier estimations of total volatile bases was a solution of potassium carbonate, as suggested by Venkataraman and Chari (1950). The effect of this alkali on the values of T.V.N. obtained from aqueous extracts of varying concentrations from different samples of cured fish has been studied. The results which are averages of triplicate analyses are tabulated as shown in Table I. It may be noted that with increasing concentra-

tions of the muscle in the aqueous extract, the time of distillation and the concentrations of the alkali have been increased proportionately. The

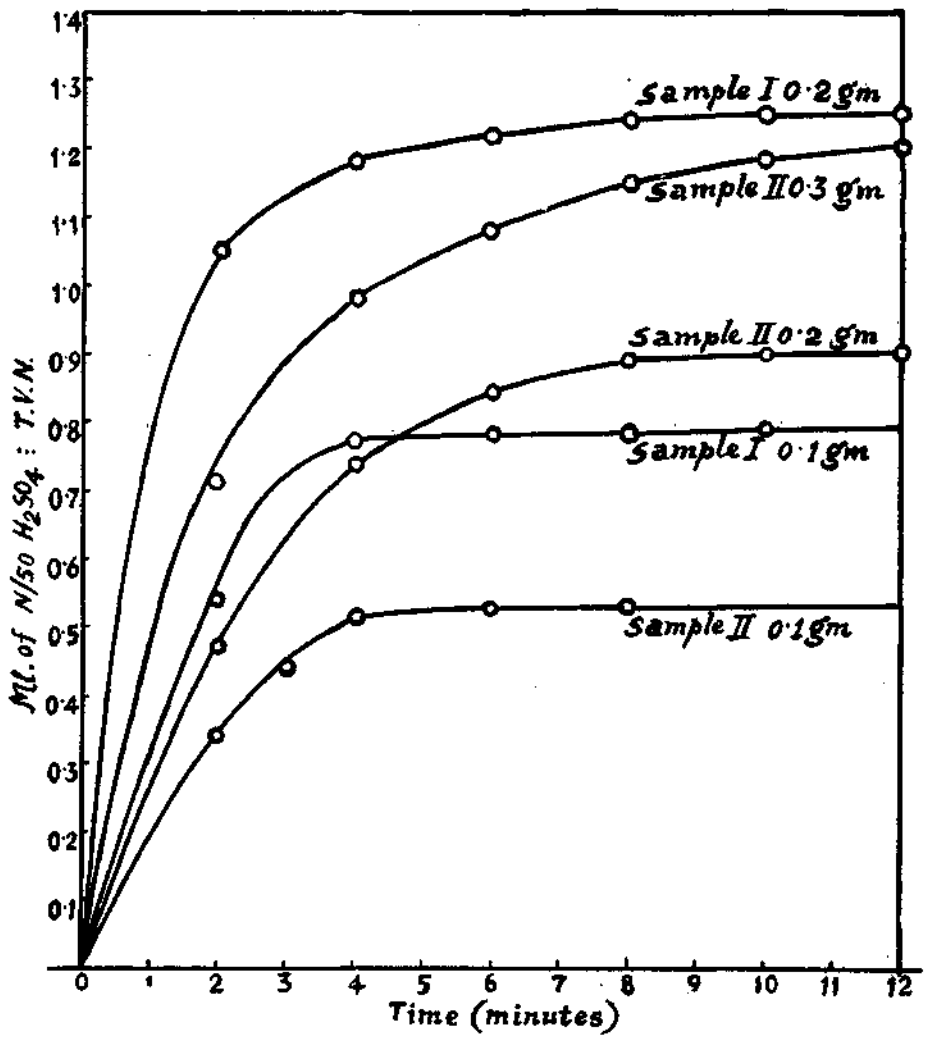

FIG. 1. T.V.N. values during different periods of distillation.

results indicate that the values of T.V.N., obtained when the extracts are distilled with saturated potassium carbonate solution, are not strictly proportional to the weights of the fish contained in the extracts. This is found to be true in the case of all the six samples, *viz.*, *Cybium*, *Caranx* sp., *Chirocentrus* sp., Sardine 1, Sardine 2 and Sardine 3.

Use of sodium borate and sodium hydroxide.—Having seen that the values of T.V.N. are not strictly proportional to the weight of the fish muscle

TABLE I

Values of T.V.N. from aqueous extracts of dry cured fish using saturated potassium carbonate solution as alkali

(Values expressed as ml. of N/50 sulphuric acid.)

Wt. of fish in extract g.	Period of distillation mts.	Vol. of alkali added ml.	ml. of N/50 H_2SO_4					
			Cybium	*Caranx* sp.	*Chiro-centrus*	Sardine 1	Sardine 2	Sardine 3
0·05	4	0·5	0·34	0·25	0·57	..	0·38	0·33
0·10	4	1·0	0·60	0·47	0·90	1·10	0·69	0·57
0·15	6	1·5	0·81	0·58	1·25	1·48	0·93	0·67
0·20	8	2·0	1·16	0·75	1·57	1·87	1·41	0·79
0·25	10	2·5	1·45	0·94	2·05	2·28	1·41	1·00
0·30	12	3·0	1·70	1·00	2·23	..	1·56	1·16

in the extract, when potassium carbonate was used as the alkali, the experiments were repeated using two other alkalies, sodium borate (saturated solution) and sodium hydroxide (40% aqueous solution). Table II gives the average values of T.V.N. from the aqueous extracts containing different weights from different varieties of fish when distilled in the presence of the three alkalies. Extracts of identical quantities of the same fish were distilled separately in duplicate series and the corresponding values of T.V.N. recorded.

The values of T.V.N. obtained with respect to two fish samples, *viz.*, *Cybium* and Sardine with the three different alkalies are plotted into graphs (Figs. 2 and 3).

The analytical data recorded above reveal certain interesting facts. Given uniform duration of distillation the values of T.V.N., obtained by using potassium carbonate and sodium borate, are on the average not proportional; the divergence from the normal expected values being more pronounced with the former alkali than with the latter. With sodium hydroxide it has been observed that the values for T.V.N., although comparatively higher always, are almost proportional to the quantity of the fish muscle present in the extracts. In this connection it may also be noted that the volume of sodium hydroxide was increased corresponding to the increase in

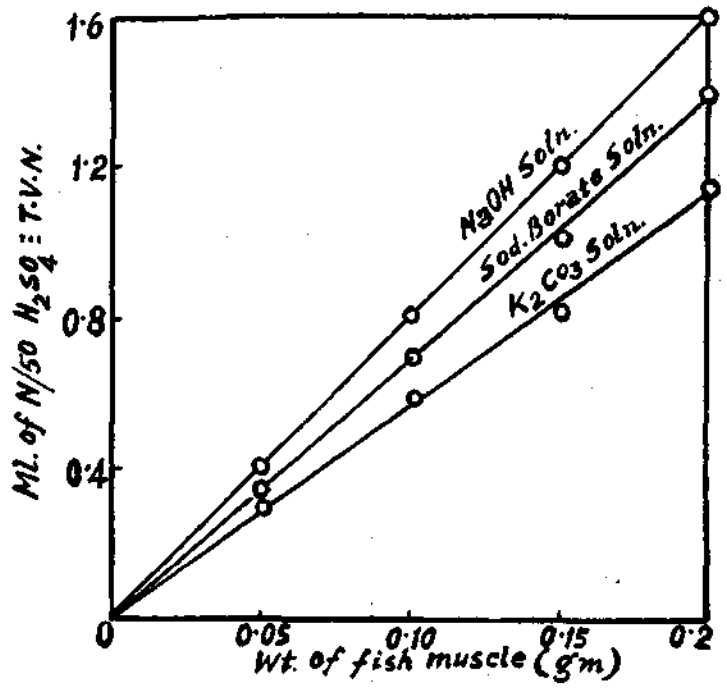

FIG. 2. T.V.N. values of a sample of dry cured *Cybium*, with different alkalies.

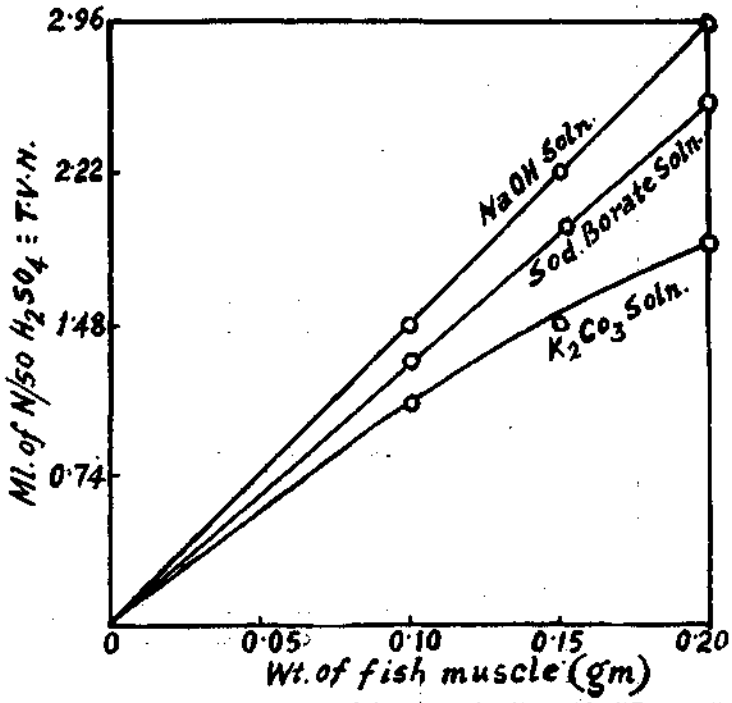

FIG. 3. T.V.N. values of a sample of dry cured Sardine with different alkalies.

TABLE II

Values of T.V.N. from aqueous extracts of cured fish using three different alkalies, viz., *A. Sodium borate (saturated solution), B. Potassium carbonate (saturated solution), C. Sodium hydroxide* (40%)

(Expressed in terms of ml. of N/50 sulphuric acid.)

Wt. of sample in the extract in g.	Period in mts.	Alkali used in ml.	ml. of N/50 H_2SO_4											
			Cybium sp. dry cured			Sardine dry cured			*Thynnus* sp. dry cured			*Caranx* sp. wet cured		
			A	B	C	A	B	C	A	B	C	A	B	C
0·05	4	0·5	0·35	0·34	0·40	..	..	..	0·33	..	..	0·55	..	..
0·10	4	1·0	0·69	0·60	0·80	1·30	1·10	1·47	0·65	0·54	0·80	1·08	1·00	1·21
0·15	6	1·5	1·00	0·81	1·21	1·45	1·48	2·22	0·96	0·68	1·18	1·60	1·41	1·81
0·20	8	2·0	1·40	1·16	1·59	2·55	1·87	2·95	1·26	0·86	1·56	2·07	1·80	2·40
0·25	10	2·5	1·65	1·45	..	3·18	2·28	3·74	1·55	1·03	..	2·60	2·25	3·03
0·30	12	3·0	1·96	1·70	..	..	..	..	1·85	..	..	..	..	..

the quantity of material used for distillation. In another series of experiments the volume of alkali was kept constant and the values for the T.V.N. determined on extracts containing varying quantities of fish muscle. The results (Table III) indicated that here again the values obtained were proportional to the concentration of the extract, even though the volume of NaOH added was kept constant throughout.

TABLE III

T.V.N. values from aqueous extracts of dry cured fish using sodium hydroxide as alkali

Sl. No.	Weight of sample in extract in g.	Volume of alkali used in ml.	T.V.N. (ml. of N/50 acid)
1	0·05	1·0	0·20
2	0·10	1·0	0·40
3	0·15	1·0	0·59
4	0·20	1·0	0·81
5	0·25	1·0	1·00
6	0·30	1·0	1·21

But it has been suggested by Beatty and Gibbons (1937) that the use of sodium hydroxide cannot be recommended, as distillation of total volatile bases with this alkali would produce basic substances due to its action on the muscles during prolonged distillation.

Among the other alkalies sodium borate can always be considered as a better reagent for the determination of total volatile nitrogen content. It is also a milder alkali than the others and hence the error due to possible decomposition of the muscle protein also is avoided.

Use of alcoholic (96%) *and aqueous trichloracetic acid extracts.*—The study of the effect of the three alkalies on the values of the total volatile bases for different concentrations of aqueous muscle extract has been extended on similar lines to two other extracts, *viz.*, ethyl alcohol (96%) and trichloracetic acid (10% aqueous solution). The average values of T.V.N. are tabulated in Table IV. The table shows that the alcoholic extracts always yield the

TABLE IV

Values of T.V.N. for extracts with water, 96% ethyl alcohol and trichloracetic acid

(Alkalies used—A. Sodium borate, B. Potassium carbonate and C. Sodium hydroxide.)

Nature of extract	Wt. of sample in the extract in g.	Period of distillation in mts.	Volume of alkali used in ml.	ml. of N/50 sulphuric acid								
				Caranx sp. wet cured			*Serranus* sp. dry cured			*Mugil cephalus* dry cured		
				A	B	C	A	B	C	A	B	C
Water extract ..	0·05	4	0·5	0·55	..	..	..	..	..	..	..	..
	0·10	4	1·0	1·08	1·00	1·21	0·37	0·33	0·40	0·87	0·73	1·00
	0·15	6	1·5	1·60	1·41	1·81	0·53	0·45	0·59	1·28	1·05	1·49
	0·20	8	2·0	2·07	1·80	2·40	0·72	0·60	0·81	1·68	1·33	2·05
	0·25	10	2·5	2·60	2·25	3·03	0·90	0·76	1·02	2·15	1·70	..
	0·30	12	3·0	..	..	..	1·06	0·87	..	2·47	..	..
Alcohol extract ..	0·05	4	0·5	0·53	..	..	..	..	..	..	..	..
	0·10	4	1·0	0·84	0·75	0·95	0·32	0·30	0·39	0·71	0·67	0·84
	0·15	6	1·5	1·24	1·16	1·43	0·48	0·42	0·59	1·00	0·95	1·29
	0·20	8	2·0	1·63	1·40	1·90	0·66	0·56	0·78	1·35	1·29	1·69
	0·25	10	2·5	2·00	1·80	2·35	0·79	0·68	0·95	1·65	..	2·13
Trichloracetic acid extract ..	0·10	4	1·0	1·25	1·20	1·36	0·51	..	0·53	0·98	0·87	1·18
	0·15	6	1·5	1·83	1·65	2·05	0·75	..	0·80	1·43	1·25	1·75
	0·20	8	2·0	2·46	2·20	2·70	1·03	..	1·09	1·92	1·63	2·38
	0·25	10	2·5	3·05	2·86	3·42	1·24	..	1·36	2·38	1·92	3·0

lowest values of T.V.N. for all samples examined, and trichloracetic acid extract yields the maximum. It is also observed that for the same extract containing a definite weight of the muscle, when distilled under identical conditions, values of T.V.N. as a rule increase in the following order: potassium carbonate, sodium borate and sodium hydroxide.

Summary and Conclusions

The values of total volatile nitrogen obtained from three extracts of different samples of cured fish when distilled under uniform conditions in presence of the alkalies—sodium borate, potassium carbonate and sodium hydroxide have been compared. Experiments were carried out with a view to find out the extent of variation in the values of T.V.N. with the increase in the concentration of the fish muscle in each of the extracts on distilling with these alkalies. The results of these experimental investigations point out that for T.V.N. determination, it is preferable to prepare an aqueous extract of the fish sample wherever possible and to use sodium borate as alkali. The advantage with alcohol extract, however, is that the proteins are precipitated and a clear solution remains.

Acknowledgement

We are indebted to Dr. N. K. Panikkar, Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp, for the keen interest he has taken during the course of the work.

References

Beatty, S. A. and Gibbons, N. E. 1937 — *Journ. Biol. Bd., Canada*, **3** (1), 77–91.

Collins, V. K. 1938 .. — *Prog. Rep. Atl. Biol. Station*, **23**, 6–9.

Conway, E. J. and Byrene. 1933 — *Biochem. Journ.*, **27**, 419–39.

——— and O'Malley, E. 1942 — *Ibid.*, **36**, 655.

Hess, F. 1941 .. — *Prog. Rep. Atl. Biol. Station*, **30**, 11–12.

Tarr, H. L. A. and Phyllis, W. Ney. 1949 — *Prog. Rep. Pac. Coast Station*, **78**, 11–13.

Velankar, N. K. 1952 .. — *Journ. Sci. Industr. Res.*, **11 A**, 359–60.

Venkataraman, K. and Chari, S. T. 1950 — *Proc. Ind. Acad. Sci.*, **31** (1) **B**, 54.