AMMONIA AND TRIMETHYLAMINE IN FISH

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By N. B. Dreyer

Products of decomposition of animal proteins are numerous. There are several well-known ones such as ammonia, which may be derived from autolytic and putrefactive changes. Other compounds due to bacterial changes fall into the class of substituted radicles in the ammonia molecule: such are mono- di- and trimethylamine. In addition, there are present other substances, such as sulphuretted hydrogen, indole, skatole, putrescine and cadaverine.

Ammonia and Trimethylamine were especially investigated. The amounts of trimethylamine were used as an index of putrefaction, since it is not formed from amino acids, but is produced by decomposition of choline and allied quarternary bases (Barger I). It is produced by Proteus vulgaris and by Bacterium prodigiosum from choline and lecithin. Since lecithin is present in all animal cells, it is evident that on keeping such material, trimethylamine can be produced easily by either bacterial action or by autolysis. It has been established, however, that no enzymes of the nature of pepsin or trypsin act on lecithin. Proteolytic enzymes, present in tissues, will not therefore cause a production of trimethylamine. When lecithin breaks up, therefore, it must be due to bacteria acting either on lecithin or choline, which is derived from lecithin.

Ammonia is easily produced by deaminating enzymes or by bacteria.

Methods of Estimation (Barger, P.124 II)

The fluid or emulsion of tissue is made alkaline with Magnesium oxide and steam-distilled. Caustic soda and sodium carbonate are avoided as these are too strongly alkaline, and during the distillation give rise to large amounts of ammonia and trimethylamine, a fact noted by previous investigators and confirmed during the present investigation.

The bases were absorbed in standard sulphuric acid. The H_2SO_4 was then neutralised with standard caustic soda, using phenolphthalein as indicator. Excess of neutral formol was added and again titrated to a pink color. This gives the amount of ammonia present. To estimate the trimethylamine the solution $N(CH_3)_3$ and $(CH_2)_6N_4$, the latter formed by ammonia and formalin interacting, is strongly acidified with concentrated hydrochloric acid and boiled down to one-third its original volume. It is again distilled in steam, after making the reaction alkaline with magnesium oxide. The bases are absorbed in standard sulphuric acid which is titrated with standard alkali, using methyl orange as indicator. This gives the total amount of bases. The trimethylamine is then estimated by difference. In all cases lead acetate was added to fix the H_2S or allied sulphur compounds, before distilling. In the process mono- and di- methylamine were not especially looked for, although they are supposed to give fish their peculiar "fishy" odour.

At first extracts of fish muscle were used, the ratio of water to fish muscle being 1 cc. water to 1 gram of muscle. Later on definite quantities of muscle were minced and the bases extracted by distillation. This method gave better results since all the bases could be extracted, whereas in the first process it was uncertain if equilibrium was established between the minced muscle and the surrounding water. The mixture of minced muscle and water was allowed to stand for two hours before filtering and distilling.

Types of Fish Used

Attention was just paid to smoked fish, since this variety is widely used as an article of diet, and in many instances is kept for several days at room temperature before consumption. The bases were determined in fresh fish immediately after brining: this served as a standard to judge the changes taking place in the smoked fish. When the smoking process was over, the bases were again determined immediately afterwards, and then at intervals of two days, until the fish were regarded as unfit for human consumption. These fish were judged on the following points: Presence of mould, slime, more particularly the odour given out. It should be noted that the sense of smell is probably the most delicate test for putrefaction, where protein is spoiling in the presence of air. Chemical tests, while giving valuable information, cannot as yet replace those ordinarily used by the fisherman, namely the smell and appearance of the fish.

Table I. NH_3 and $N(CH_3)_3$ in smoked fish extracts.

N.B. The figures represent cc. N/10 of NH_3 and $N(CH_3)_3$ in all tables.

	NH_3	$N(CH_3)_3$
1st. day	7.2	10.8
3rd. day	19.4	24.9
6th. day	21.6	25.55

In another experiment the following values were found.

	NH_3	$N(CH_3)_3$
2 days after smoking	3.82	4.08
4 " " "	9.7	10.2

The brine used was of 55% saturation, and the fish were left in it for one hour. A "formalinised" fish from the same group, on the other hand, gave the following figures, eleven days afterwards NH_3 6.47 cc. $N(CH_3)_3$ 11.35 cc. This fish was not smoked, but exposed to large quantities of air, into which formalin vapour was passed. The amount of neutral formol used was 10 cc., the amount of air amounted to several thousand cubic feet. It will be seen from the above figures that a smoked fish on the 4th day had changed almost as much as the formalinised fish had on the 11th day. On this particular fish no odour of formalin could be detected. Some of it was prepared for lunch. It gave rise to no poisoning and its flavour was quite as good as that of the ordinary smoked variety.

The formaldehyde was "neutralised" by combination with the NH_2 groups of the proteins, but before this happened it must have exerted its antiseptic action.

It should be noted that the different amounts of ammonia and trimethylamine found in different "fresh" fish, depends upon the time the fish had been kept on ice after being caught. In one lot this may be one day, in other two or three days.

Effects of brining alone, and brining combined with smoking were compared. The experiment was carried out as follows: Four fish were brined for the same length of time and then divided into two groups of two each. One lot was dried only until the surface no longer showed any trace of moisture and presented a definite pellicle. The others were similarly treated, but were smoked in addition for 6 hours. Tables in Table II.

Table II.

	Smoked Fish		Unsmoked Fish	
	NH_3	$N(CH_3)_3$	NH_3	$N(CH_3)_3$
3rd day	2.6	3.8	7.2	7.2
5th day	4.5	11.66	35.10	24.70
7th day	9.90	28.37	These fish had become rotten	

The figures indicate that smoking acts as a preservative. Even after three days the change is very noticeable, while on the seventh day there is no doubt whatever as to the action of smoke.

Action of Salt Alone

Four fish were placed in saturated brine for twenty-four hours. They were sun-dried on the following day, and then placed in a room, where the temperature varied from 55-60°F. Six days after brining, no putrefaction could be detected. Extracts of the fish tested for ammonia and trimethylamine gave the following results. (Table III)

Table III.

	NH_3	$N(CH_3)_3$
6th day after brining	5.05	6.05
8th " " "	5.20	6.79
13th " " "	-	11.47

On the 13th day the fish were certainly showing signs of spoiling, for moulds had made their appearance.

Brines of Different Concentrations

(1) saturated salt solution plus slight excess of salt.

(2) saturated brine.

(3) 60% saturated brine.

Ordinary preserving jars with stoppers were used, so as to prevent any evaporation of water. It was found that 450 grams of fish and 330 cc. of brine filled a jar, and this proportion of fish to brine was used for several experiments. At the end of the experiment the amount of salt in solution was much lower than at the commencement, for water was withdrawn from the fish. In (1) however enough salt was present to keep the solution saturated. Estimations of NH_3 and $N(CH_3)_3$ six days later gave the following amounts in the different brines. (Table IV)

Table IV.

Weight of fish	Original Volume of brine	Brine recovered	NH_3	$N(CH_3)_3$
(1) 450 grams	330 c.c.	500 c.c.	6.30	2.09
(2) 450 grams	330 c.c.	400 c.c.	13.72	7.32
(3) 450 grmas	330 c.c.	-	40.50	27.10

No estimations were carried out on the fish muscle. In later work, however, total amounts of NH_3 and $N(CH_3)_3$ were measured in the brine and fish. No doubt is felt as to the better preserving qualities of strong brines. Only in jar (1) was no bad odour detected: jar (2) gave out a slight odour and jar (3) on opening gave the usual putrefaction smell, but further, gases under pressure escaped, and the fish had become soft and pulpy. The next series give a better indication of the changes taking place with brines of different concentrations. Estimation at regular intervals, not only of ammonia and trimethylamine were carried out but also of the amino-introgen.

The brines were of the following strengths.

(1) Saturated some solid salt

(2) Saturated brine

(3) 80% saturated

(4) 60% saturated

The batch of fish which was preserved in these brines was quite fresh. The figures obtained from extracts made immediately after reaching the laboratory gave amounts of NH_3 per 100 c.c., 2.1 c.c. $N/_{10}$ and $N(CH_3)_3$ 2.7 c.c. $N/_{10}$.

Table V.

	No. of days after brining	NH_2 Nitrogen	NH_3 %	$N(CH_3)_3$ %
No. 1	3	14.0	2.0	3.0
	5	14.0	2.6	4.0
	9	20.0	2.8	5.3
	13	22.0	5.2	5.6
No. 2				
	3	16.5	-	6.0
	5	22.0	2.8	-
	9	30.0	-	6.8
	13	36.0	7.0	7.8
No. 3				
	3	22.0	5.2	8.6
	5	28.0	5.6	-
	9	39.0	10.4	18.0
	13	48.0	14.8	21.6
No. 4				
	3	20.0	4.8	13.6
	5	31.2	8.6	17.4
	9	40.0	11.2	-
	13	50.0	14.0	23.6

These findings apply to haddock.

It will be seen that the ammonia and trimethylamine mount only slowly in the salt solutions of high concentration, while in the weaker solutions the rise is more rapid. The amount of protein in solution is also much greater in the weaker brines. Herring treated in the same way gave similar results.

Brines of the following strengths were used:- (Table VI)

(1) saturated brine + solid salt 330 c.c.

(2) saturated brine 330 c.c.

(3) 80% saturated brine 330 c.c.

Weight of muscle in each case 450 grams.

Table VI.

	Time in brine	N H_2 Nitrogen in brine	NH_3	$N(CH_3)_3$
No. 1				
	2 days	12.8	1.0	-
	4 "	20.0	1.6	7.6
	7 "	24.0	4.2	11.2
	11 "	32.0	8.0	10.8
	14 "	32.0	9.0	10.8
No. 2				
	2 "	12.8	1.4	1.6
	4 "	20.0	3.2	7.6
	7 "	32.0	6.0	12.0
	11 "	40.0	12.2	14.0
	14 "	44.0	15.4	18.0
No. 3				
	2 "	13.6	1.6	8.2
	4 "	28.0	8.0	13.4
	7 "	42.0	12.0	25.2
	11 "	48.0	17.6	-
	14 "	85.0	47.0	24.2

The ammonia and trimethylamine give values similar to those previously found with haddock and cod. Since it was difficult to determine the amounts of NH_3 and $N(CH_3)_3$ present in muscle, the following method was adopted. 50 grams of fish muscle were immersed in 50 cc. brine of different strengths and the NH_3 and $N(CH_3)_3$ determined in the brine and the muscle . (Table VII). Each bottle was corked to prevent escape of gases and to prevent NH_3 absorption from the air. They were all kept at a temperature varying from 60-65° F.

The series were made up as follows:-

(1) 50 grams fish +50 c.c. saturated brine

(2) 50 " " +50 c.c.80% " "

(3) 50 " " +50 c.c.60% " "

Table VII.

	Days after brining	Weight of muscle	NH_3	$N(CH_3)_3$
No.1	3	50 grams	14.6	-
	5	"	20.6	8.3
	9	"	26.2	-
No.2				
	3	"	12.4	-
	5	"	22.9	9.6
	9	"	33.0	11.8
No.3				
	3	"	17.5	21.7
	5	"	39.0	47.4
	9	"	45.3	47.9
	Control fish on first	day "	9.4	7.8

The values for muscle + brine show no difference from those found previously for brines alone.

Effect of Temperature Alone

To determine the influence of temperature only on putrefactive and autolytic changes, fish were kept at different temperatures. No salt or other preservative was added. The following temperatures were selected:-

(1) 32-35°F. (2) 45-50°F. and (3) 62-65°F.

The first and third were easily maintained, but in the second it was more difficult. In spite of this, however, it was possible to get a fair idea of the relative rate of change.

Table VIII. Extracts of fish 1 gram of muscle: 1 c.c. H_2O

	Days	N H_3	N$(CH_3)_3$
No.1 32-35°F.	2	2.54	0.16
	5	3.08	0.65
No.2 45-50°F.	2	2.92	0.68
No.3 62-65°F.	2	5.0	6.6
	5	15.18	9.02

A second set gave the following results:-

Table IX.

	Days	N H_3	N$(CH_3)_3$
No. 1	1	0.68	0.67
	3	0.66	1.19
	4	1.12	1.42
No. 2	1	1.32	2.09
	3	2.23	6.98
	4	-	-

Table IX.

	Days	NH_3	$N(CH_3)_3$
No. 3	1	-	2.33
	3	14.97	15.08
	4	24.8	20.3

The differences observed on the first days of each experiment were due to the condition of the fish. In the first case they were freshly caught and put in containers within half an hour after killing. In the second the fish were bought and were presumably dead for more than one day. The figures given above make it clear that basic compounds such as NH_3 and $N(CH_3)_3$ appear in greater quantities the higher the temperature.

The values obtained for NH_3 and $N(CH_3)_3$ in fish under-different treatments show that both substances increase in quantity from day to day. Whether these substances are themselves broken down by bacteria, to liberate nitrogen gas, was not determined. The stoppered jars which were used always showed the presence of large quantities of gas under pressure. This was undoubtedly of mixed composition and it is possible the nitrogen derived from protein sources was present. However at present it is impossible to say that NH_3 is entirely due to autolysis and $N(CH_3)_3$ to putrefaction. While the latter is undoubtedly due to bacterial action, it seems most probable that NH_3 is derived from two sources, namely autolysis and putrefaction. The source of $N(CH_3)_3$ is lecithin and allied substances. Since these are present in limited quantities, it might be expected that the amounts of $N(CH_3)_3$ can rise to a certain maximum, when all the lecithin has been decomposed. In no instance was this found to be the case, unless the periods of observation were not long enough for arriving at the maximum.

Conclusions

Fish kept under conditions favourable for spoiling, all show increases in the amounts of NH_3 and $N(CH_3)_3$. High temperatures and brines with low salt content, lead to quick spoilage. Low temperatures, brines with high salt content and lightly "formalinising" have a marked preservative action. Smoked fish show only slight changes for the first few days, but thereafter deteriorate rapidly. High salt content does not completely prevent bacterial action, as shown by the gradual rise in the amounts of $N(CH_3)_3$ in the brine.

Bibliography

I. Barger - The Simpler Natural Bases
II. Barger - The Simpler Natural Bases Page 124