

Amines in Fish Muscle

II. Development of Trimethylamine and Other Amines

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ABSTRACT

Relatively large amounts of nonvolatile higher amines are formed during proteolysis of fish muscle by bacteria. This follows closely the formation of trimethylamine. Fresh fish contain 0.2 mg. nitrogen per 100 g. muscle as trimethylamine and about 0.1 mg. nitrogen per 100 g. as dimethylamine. Trichloroacetic acid and formaldehyde solutions completely extracted trimethylamine from fish and satisfactory recovery of added trimethylamine was obtained.

The simple, rapid measurement of fish quality has always been one of the vexing problems of the fishing industry. Surface pH determination provides a rapid index for stored fillets, but is not satisfactory for round fish or for fillets immediately after cutting, since the surface pH depends on the accumulation of the products of bacterial action in the exposed surface layer, and the test thus measures only the spoilage which has taken place after filleting (Dyer, Sigurdsson and Wood 1944). The trimethylamine level provides the best indication of bacterial deterioration in sea fish of any of the various spoilage effects investigated to date. The studies of Beatty and others (Beatty and Gibbons 1937, Beatty and Collins 1939, Watson 1939) have shown that the bacteria first utilize the lactic acid and sugar in the tissues, simultaneously reducing the trimethylamine oxide present to the odoriferous volatile base trimethylamine. This occurs at first only on the surface tissue, the locus of bacterial growth, so that in an average sample of muscle, the rise in trimethylamine is gradual (Wood, Sigurdsson and Dyer 1942). Thus trimethylamine production constitutes a warning of incipient spoilage by bacterial action.

Dimethylamine and monomethylamine are also formed in small amounts by bacterial action (Beatty and Collins 1940). Shewan (1938) has proposed the measurement of dimethylamine as a spoilage test.

Histamine, phenylethylamine, tyramine, indole and other amines are formed during protein putrefaction, and Geiger (1944) has recently proposed a spoilage test based on the determination of histamine.

The development of the colorimetric method of estimation of trimethylamine (Dyer 1945) resulted in a study of the amines developed over the spoilage curve, a comparison of various extraction methods, and the establishment of the trimethylamine content of fresh fish.

IN COD MUSCLE BREI

A suspension of minced fresh cod muscle in an equal weight of distilled water was incubated at 20°C. Samples were withdrawn at intervals for analysis. The proteins were precipitated by the addition of 10 ml. water and 20 ml. of 10 per cent trichloroacetic acid to the 10 ml. of muscle brei. Trimethylamine was determined by both the picric acid colorimetric method and the Conway procedure (Beatty and Gibbons 1937). The results are shown in table I.

TABLE I. Amine formation in cod muscle brei at 20°C.

| Incubation time (hours) | Trimethylamine content (mg. nitrogen per 100 g. muscle) | | |
|-------------------------|---|--------|------------|
| | Colorimetric | Conway | Difference |
| 12 | 3.0 | 4.0 | .. |
| 18 | 11.0 | 10.2 | .. |
| 20 | 18 | 18 | .. |
| 22 | 33 | 31 | .. |
| 23 | 38 | 39 | .. |
| 25 | 60 | 62 | .. |
| 26 | 80* | 75 | 5 |
| 27 | 90 | 77 | 13 |
| 38 | 125 | 80 | 45 |
| 45 | 155 | 80 | 75 |
| 50 | 165 | 80 | 85 |
| 64 | 210 | 83 | 127 |
| 86 | 250 | 87 | 163 |
| 94 | 300 | 95 | 205 |
| 110 | 350 | 110 | 240 |

*First putrid odour.

The reduction of trimethylamine oxide by the bacterial enzymes follows a logarithmic curve as shown in figure 1.

Thus the reaction is of the first order, from the slow initial stages right through to the completion of the reduction. The results of the two methods of trimethylamine determination agree very well up to the point where the trimethylamine production is almost complete, a level of about 60 mg. nitrogen per 100 g. tissue in the sample under examination. At 26 hours incubation time, the Conway value has reached 75 while the colorimetric method gives 80 mg. nitrogen per 100 g. fish. Hereafter the difference increases at a very rapid rate, the colorimetric value being greater by 240 mg. per 100 g. after 110 hours, as shown in the table. The point of first production of these nonvolatile amines corresponds with the first appearance of the odours associated with protein putrefaction. Many amines are formed during bacterial breakdown of protein, chiefly due to decarboxylation, as discussed more fully by Schmidt (1938, p. 236), and Gale (1940). Putrescine, cadaverine, tyramine, phenylethylamine, and histamine are examples

of those formed. Some of these have been shown to react with the picric acid reagent but they are not sufficiently volatile to be included in the distillation method (Dyer 1945). Thus the production of trimethylamine is closely followed by decomposition or putrefaction of the protein.

This confirms the conclusion reached by Beatty and Collins (1939), who demonstrated the deamination of amino acids in cod muscle press juice during advanced spoilage and who suggested the possible formation of amines by the decarboxylation of amino acids.

IN STORED COD MUSCLE

Very similar results were obtained on cod muscle. Fresh cod fillets, two or three hours out of the water, were stored at 2°C. Samples were removed at intervals and extracted with trichloroacetic acid, a 25 g. minced sample being shaken

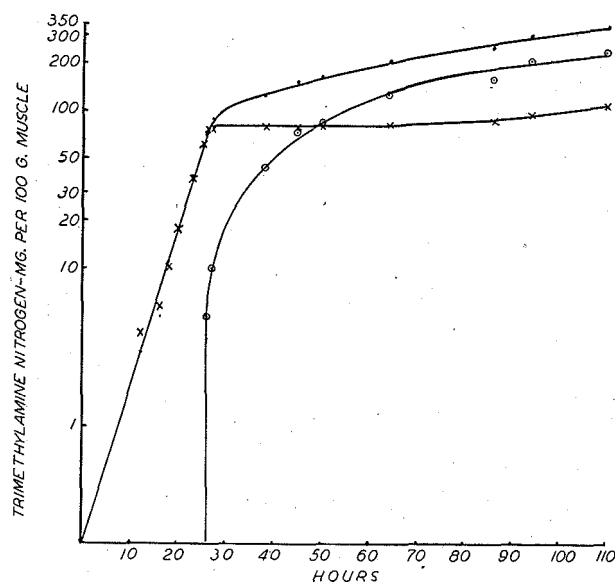


FIGURE 1. Amine production in cod muscle brei.

with 25 ml. water and 50 ml. of 10 per cent trichloroacetic acid. The filtered extract was analysed by the colorimetric and Conway methods in triplicate, with results as shown in table II.

The two methods are in excellent agreement, and development of higher amines did not occur until the fish was quite putrid. Results from the analysis of a large number of samples of spoiled fish showed that no significant development of these amines occurred until the fish had reached a trimethylamine content of about 60 to 80 mg. nitrogen per 100 g. muscle. Organoleptically, cod and haddock muscle is considered undesirable if the trimethylamine content is over 10 or 20 mg. nitrogen per 100 g. tissue so that the measurement of these amines is of no value as an indicator of spoilage, and they do not affect the results obtained by the colorimetric method over the useful range.

Since the production of these amines occurs only during protein breakdown, when the product has reached an advanced state of decomposition, the content of these nonvolatile amines might be a useful indication of protein putrefaction in meats as well as in fish. This is of particular significance since many of the amines produced are quite poisonous, exhibiting marked sympathomimetic activity.

The development of trimethylamine in fish muscle is not logarithmic, since the bacteria act only on the surface and a composite sample is analysed. No development of higher amines was detected at the lower values even though some protein decomposition would be expected in the highly contaminated surface area. Analysis of surface samples would possibly show such a reaction.

TABLE II. Development of amines in cod muscle stored at 2°C.

| Time of storage (days) | Trimethylamine content (mg. nitrogen per 100 g. muscle) | | | |
|------------------------|---|----------------|--------|----------------|
| | Colorimetric | | Conway | |
| 0 | 0.19 | (± 0.02) | 0.23 | (± 0.04) |
| | 0.22 | (± 0.07) | 0.22 | (± 0.06) |
| 2 | 0.36 | (± 0.05) | 0.30 | (± 0.1) |
| 3 | 0.33 | (± 0.14) | 0.40 | (± 0.2) |
| 4 | 0.31 | (± 0.07) | 0.34 | (± 0.1) |
| 5 | 0.81 | (± 0.08) | 0.80 | (± 0.1) |
| 6 | 2.7 | (± 0.2) | 2.7 | (± 0.1) |
| 7 | 32.5 | (± 4) | 31.5 | (± 3) |
| 12 | 119. | (± 3) | 94. | (± 1) |

Samples of fillets, smoked fish and salt fish are not infrequently found on the market with trimethylamine values of 60 mg. nitrogen or over although only rare cases of food poisoning from decomposed fish are encountered. The presence of the easily recognized trimethylamine base should serve as a warning of incipient protein putrefaction.

TRIMETHYLAMINE AND DIMETHYLAMINE IN FRESH FISH

In the above experiment and in numerous other analyses of fresh cod and haddock muscle made in the laboratory, fresh muscle was found to contain approximately 0.18 to 0.20 mg. nitrogen per 100 g. muscle as trimethylamine. Fish only a few hours out of the water gave the same value as those stored for longer periods but in which trimethylamine oxide reduction had not begun. Thus the enzymic equilibrium in sterile fish muscle tissue greatly favours the oxide form, the ratio of trimethylamine oxide to trimethylamine being about 100 to 0.2.

Shewan (1939) suggests that the apparent trimethylamine obtained by the use of the Beatty and Gibbons' method on fresh cod during the initial stages of bacterial breakdown is actually dimethylamine. Dimethylamine gives only 20 per cent as much colour as trimethylamine in the colorimetric method (Dyer 1945). Thus the close agreement between the colorimetric and distillation

methods is evidence that very little dimethylamine is formed since it is determined in the distillation method. Using the method given below, fresh cod was found to contain about 0.1 mg. dimethylamine nitrogen per 100 g. muscle. Thus the colorimetric procedure would show 0.02 mg. nitrogen per 100 g. muscle as trimethylamine due to the dimethylamine present or only 10 per cent of the trimethylamine found. A similar relation holds for the early part of the spoilage curve.

DIMETHYLAMINE PRODUCTION IN COD MUSCLE

Dimethylamine was first determined in fish by Reay (1938) and later by Shewan (1938, 1939) and Beatty and Collins (1940) who used the method of Dowden (1938) for dimethylamine in urine. We experienced many difficulties in the application of the method to fish tissue and a study of the reaction was made using the photo-electric colorimeter. The method adopted was as follows. An aliquot of sample solution or trichloroacetic acid extract of fish containing 0.001 to 0.006 mg. dimethylamine nitrogen is diluted to 10 ml., 1 ml. of copper-ammonia reagent (20 g. ammonium acetate and 0.2 g. copper sulphate dissolved in 30 ml. water added to a solution of 10 g. sodium hydroxide in 25 ml. water and 20 ml. concentrated ammonium hydroxide all diluted to 100 ml.) is added, followed by 10 ml. of 5 per cent solution of carbon disulphide in benzene. The mixture is heated in a water bath at 40 to 50°C. for 5 minutes. The tubes are tightly stoppered and shaken rapidly in a shaking machine for 5 minutes. One ml. of 30 per cent acetic acid is added and shaken until the solution is clear, about 10 or 20 times. The benzene layer is decanted, dried by shaking with about 0.4 g. anhydrous sodium sulphate and the yellow colour of copper dimethyldithiocarbamate measured in the photoelectric colorimeter using a filter with maximum transmission at 4400 ångström units. The blank is made with distilled water instead of the sample solution. The colour is stable and is proportional to the concentration within the limits given as shown in figure 2. Complete recovery of dimethylamine added to fish extracts was obtained.

Results on fresh cod and haddock gave values of about 0.1 mg. nitrogen per 100 g. This increases to about 3 or 4 with spoiled fish. The development of dimethylamine occurs early in the spoilage cycle but the values were found to be too variable for the comparison of different lots of fish. Thus it is not recommended as a spoilage test.

TRIMETHYLAMINE AS A SPOILAGE INDICATOR

Beatty (1938) has shown that at least 94 per cent of the trimethylamine in spoiling fish originates from the reduction of trimethylamine oxide. Keeping (unpub.) and Wood and Keeping (1944) have shown that a few species of bacteria, certain of which may be found on fish, can produce trimethylamine from choline in a culture medium, but only in the absence of an easily oxidizable carbohydrate source. Thus the lactic acid present in fish muscle would probably suppress the reaction. Furthermore, only a very small proportion of the bacteria contaminating fish muscle can split choline, and only traces of trimethylamine are found in fresh water fish (Lintzel, Pfeiffer and Zippel 1939, Shewan 1942).

Fletcher, Best and Solandt (1935) have shown that cod muscle contains only the equivalent of 9 mg. choline nitrogen per 100 g., so that trimethylamine production from choline is not likely to be important, and would in any case be a bacterial spoilage product.

Working with Pacific coast fish, Tarr (1940) has suggested that spoilage in fish containing trimethylamine oxide may occur without trimethylamine formation. As far as Atlantic ground fish are concerned, this is only a rare occurrence; out of more than 3,000 samples examined in the past year only one case was found where the trimethylamine was low and protein degradation was evident. Results obtained in this laboratory (F. E. Dyer unpublished) have shown that from 10 to 40 per cent of the organisms present in fish slime and spoiling fish

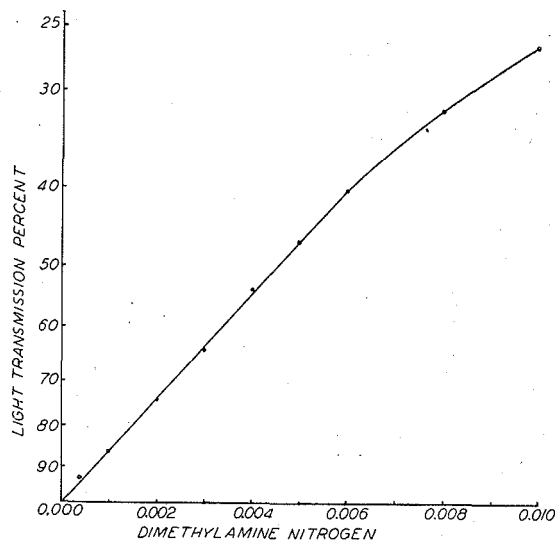


FIGURE 2. Standard curve for dimethylamine. Per cent light transmission, logarithmic scale, plotted against mg. dimethylamine nitrogen per tube.

reduce trimethylamine oxide and that these are active in the presence of other forms.

EXTRACTION METHODS

Previous methods of trimethylamine determination have used weighed samples of fish directly, or the press juice obtained by pressing out the sample. Because of its greater convenience an extraction procedure has been developed. Trichloroacetic acid extracts have been used, usually on 10 and 20 g. minced samples. The sample is mixed with an equal weight of water, followed by the addition of a 10 per cent solution of trichloroacetic acid, in amount twice that of the water added. The mixture is shaken several times over a period of a half hour or longer and is then filtered, although often an aliquot is removed from the supernatant liquid for analysis without filtration. For the inspection of large numbers of samples of frozen fish for the British Ministry of Food, a method was

developed for taking samples for shipment to the laboratory for analysis. Pint jars containing 50 ml. of a formaldehyde solution, commercial formalin diluted with an equal volume of water, were used, and an approximately 100 g. sample of the fillet to be tested was placed in the jar, which was then sealed. The mixture of sample and formaldehyde solution was weighed at the laboratory to determine the actual sample weight, 150 ml. of water was added, and the mixture pulped in the Waring Blendor. After standing a few minutes the supernatant liquid was decanted and used for the trimethylamine determination. Since the colorimetric method is more sensitive than the microdistillation technique, the use of greater dilutions is permissible than with the latter. Both the formaldehyde and trichloroacetic acid extraction methods in conjunction with the colorimetric tri-

TABLE III. Comparison of extraction methods.

| Method of extraction | Trimethylamine nitrogen found | |
|--|---|---|
| | Mg. nitrogen (av. per 100 g. muscle) | Percentage of distillation method |
| Distillation*—2 g. fish | 12.0 12.0 12.0 | 100 |
| Trichloroacetic acid 20 g. fish + 20 ml. water + 40 ml. trichloroacetic acid | 11.5 11.0 11.6 | 95 |
| 10 g. fish + 30 ml. water + 40 ml. trichloroacetic acid | 11.5 11.1 11.5 | 95 |
| Formaldehyde. 100 g. fish + 50 ml. formaldehyde + 150 ml. water | 12.0 11.2 12.0 | 97.5 |
| 50 g. fish + 50 ml. formaldehyde + 150 ml. water | 11.9 11.0 11.9 | 97 |

*Beatty and Gibbons semimicrodistillation.

methyamine determination have been used successfully on many thousands of samples over the past two years. In table III are shown the results of an experiment in which the above procedures are compared with the Beatty and Gibbons distillation method on the same well comminuted sample of cod muscle.

The three methods are in good agreement, and although the results obtained by the trichloroacetic acid extraction were not quite as high, 95 per cent of those by the direct distillation method, this is quite satisfactory for practical use in a spoilage test. Stansby et al. (1944) obtained very poor extraction of trimethylamine from fish, using trichloroacetic acid. These authors used press juice with a small volume of concentrated trichloroacetic acid solution added, and found that

considerable trimethylamine remained entrained in the voluminous protein precipitate. In the present work, 1 to 4 and 1 to 8 dilutions of the original muscle were used with satisfactory results.

Known amounts of trimethylamine added to fish muscle were recovered satisfactorily by all the above methods as shown in the experiment reported in table IV.

TABLE IV. Recovery of trimethylamine in three extraction methods.

| Method of extraction | Trimethylamine nitrogen found (mg. N per ml. extract) | | | | Trimethylamine recovered | |
|-----------------------------------|--|--------|-------|--------|----------------------------|-------------|
| | Av. | | Added | | mg. nitrogen per ml. | Per cent |
| Distillation—2 g. fish | 0.24 | 0.24 | 0.24 | — 0.24 | | |
| “ + 1 mg. N-Trimethylamine | 1.22 | 1.22 | 1.22 | — 1.22 | 1.00 | |
| | | | | | 0.98 | 98.0 |
| Trichloroacetic acid | | | | | | |
| 20 g. fish + 20 ml. water | | | | | | |
| “ + 40 ml. acid | 0.0278 | 0.0272 | — | 0.0275 | | |
| “ + 2 mg. N-Trimethylamine | 0.524 | 0.528 | — | 0.526 | 0.025 | |
| | | | | | 0.0251 | 100.0 |
| 10 g. fish + 30 ml. water | | | | | | |
| “ + 40 ml. acid | 0.0132 | 0.0147 | — | 0.0140 | | |
| “ + 2 mg. N-Trimethylamine | 0.0390 | 0.0400 | — | 0.0395 | 0.025 | |
| | | | | | 0.0255 | 102.0 |
| Formaldehyde | | | | | | |
| 100 g. fish + 50 ml. formaldehyde | | | | | | |
| “ + 150 ml. water | 0.038 | 0.042 | — | 0.040 | | |
| “ + 10 mg. N-Trimethylamine | 0.076 | 0.074 | — | 0.075 | 0.0333 | |
| | | | | | 0.035 | 105.0 |
| 50 g. fish + 50 ml. formaldehyde | | | | | | |
| “ + 150 ml. water | 0.0206 | 0.0226 | — | 0.0216 | | |
| “ + 5 mg. N-Trimethylamine | 0.0420 | 0.0400 | — | 0.410 | 0.0200 | |
| | | | | | 0.0194 | 97.0 |

The formaldehyde extract still contains considerable protein solution but this does not affect the results obtained by the colorimetric method. Similarly, direct determinations on the pickle from canned fish without protein precipitation also show no interference.

From the above results it is concluded that trimethylamine in fish muscle may be extracted by trichloroacetic acid or formaldehyde solution and the trimethylamine in the extract determined quantitatively by the picric acid colorimetric method.

SUMMARY

In the bacterial spoilage of a cod muscle brei trimethylamine is first produced followed by large amounts of higher non-volatile amines. Similar results were obtained on cod muscle. This occurred only in the advanced stages of deterioration of the muscle, at trimethylamine values of about 60 to 80 mg. nitrogen per 100 g. muscle or over. Thus food poisoning from these amines, many of which are highly active physiologically, is possible if badly spoiled fish muscle is ingested.

Fresh cod and haddock contain about 0.2 mg. nitrogen per 100 g. muscle as trimethylamine. This is not dimethylamine as has been suggested, the dimethylamine content of fresh cod and haddock muscle being about 0.1 mg. nitrogen per 100 g. Dowden's method for the determination of dimethylamine has been studied and modified for application to fish muscle extracts.

Extraction of fish muscle with trichloroacetic acid and formaldehyde solutions gave practically complete extraction of the trimethylamine present as determined by distillation methods. Satisfactory recovery of trimethylamine added to fish muscle was also obtained with each of the three methods, distillation and trichloroacetic acid and formaldehyde extraction.

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