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EFFECT OF METHODOLOGY ON THE DETERMINATION OF TOTAL VOLATILE NITROGEN AND TRIMETHYLAMINE LEVELS IN PREVIOUSLY FROZEN PACIFIC HERRING (*CLUPEA HARENGUS PALLASI*) STORED AT 2-5°C FOR UP TO 15 DAYS

G.S. Clancy, R.M. Beames, D.A. Higgs and B.S. Dosanjh

Department of Fisheries and Oceans
Science Branch, Pacific Region
West Vancouver Laboratory
West Vancouver, British Columbia
V7V 1N6 Canada

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by

G.S. Clancy, R.M. Beames[1], D.A. Higgs and B.S. Dosanjh

Department of Fisheries and Oceans
Science Branch, Pacific Region
West Vancouver Laboratory
4160 Marine Drive
West Vancouver, British Columbia
V7V 1N6 Canada

[1]Department of Animal Science, University of British Columbia
Vancouver, British Columbia V6T 1Z4 Canada

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ABSTRACT

Clancy, G.S., R.M. Beames, D.A. Higgs and B.S. Dosanjh. 1995. Effect of methodology on the determination of total volatile nitrogen and trimethylamine levels in previously frozen Pacific herring (*Clupea harengus pallasi*) stored at 2-5°C for up to 15 days. Can. Tech. Rep. Fish. Aquat. Sci. 2047: 10 p.

Ripe male herring which had been held in refrigerated seawater in a commercial fishing boat for 2 days were snap-frozen in polyethylene bags at -40°C for 24h and then held at -20°C for 90d. Storage temperature was then changed to 2-5°C with samples of fish removed daily. Each sample was analysed for total volatile nitrogen (TVN) by each of four methods and for trimethylamine (TMA) by three methods. Methods included microdiffusion with K_2CO_3 or KOH and steam distillation with NaOH for both TVN and TMA, with direct distillation with MgO also being used for TVN. Correlation between methods was high for both TVN (0.985-0.994) and TMA (0.890-0.976). For TVN, direct distillation with MgO gave consistently higher values than the other three methods. There was no significant difference between methods for TMA. For both measurements, regardless of method, there was little increase detected in levels for the first 7 days of storage. It was concluded that, for previously frozen herring stored at 2-5°C, TVN and TMA are satisfactory tests for spoilage, but only after 7 days of storage.

RÉSUMÉ

Clancy, G.S., R.M. Beames, D.À. Higgs et B.S. Dosanjh. 1995. Effet de la méthode employée sur le dosage de l'azote volatil total et de la triméthylamine chez le hareng du Pacifique (*Clupea harengus pallasi*) décongelé entreposé à 2-5 °C pendant une période allant jusqu'à 15 jours. Can. Tech. Rep. Fish. Aquat. Sci. 2047: 10 p.

Des harengs mâles matures qui avaient été maintenus en eau de mer refroidie pendant 2 jours à bord d'un bateau de pêche commerciale ont été surgelés en sacs de polyéthylène à -40 °C pendant 24 h, puis conservés à -20 °C pendant 90 jours. La température a ensuite été relevée à 2-5 °C, et des échantillons de poisson ont été prélevés chaque jour. On a mesuré dans chaque échantillon l'azote volatil total (AVT) par 4 méthodes différentes, et la triméthylamine (TMA) par 3 méthodes. Les méthodes faisaient appel à la microdiffusion en présence de K_2CO_3 ou de KOH et à la distillation à la vapeur en présence de NaOH pour l'AVT et la TMA, et à la distillation directe en présence de MgO pour l'AVT. La corrélation entre les méthodes était élevée tant pour l'AVT (0,985-0,994) que pour la TMA (0,890-0,976). Pour l'AVT, la distillation directe en présence de MgO donnait régulièrement des résultats plus élevés que les 3 autres méthodes, alors qu'on n'observait pas de différence notable entre les 3 méthodes dans le cas de la TMA. Dans les deux séries de mesures, quelle que soit la méthode employée, on notait peu d'augmentation des teneurs pendant les 7 premiers jours d'entreposage. Nous concluons que, pour du hareng décongelé maintenu à 2-5 °C, le dosage de l'AVT et celui de la TMA constituent des tests satisfaisants de la décomposition, mais seulement après 7 jours d'entreposage.

INTRODUCTION

The fishing for herring specifically for production of fish meal is prohibited in both Canada and Norway (DPA, 1988; Halland et al., 1988). In Canada, herring meal is a by-product of the roe fishery and is produced from surplus fish catches, unsold frozen stores, intact males and females without roe. The unprocessed fish are often frozen for prolonged periods when surplus catches occur and processing equipment cannot keep pace with supply (Connell, 1990).

Quality changes occur in fish before freezing, during frozen storage and after thawing. A limited number of studies have examined the spoilage of previously frozen fish after defrosting and storage at elevated temperatures (Shewan, 1961; Gould and Peters, 1971; Flick et al., 1986).

Of the chemical compounds which are measured to assess fish spoilage, total volatile basic nitrogen (TVN, TVBN) and trimethylamine (TMA) are the most common. Measurements of TVN and TMA have been criticized for lack of sensitivity during the early stages of spoilage. However, TVN and TMA are considered to be good indicators of advanced spoilage (Connell, 1990).

TVN is composed primarily of TMA and ammonia, but other substances may be present, such as dimethylamine, methylamine, and formaldehyde (Halland and Njaa, 1988), especially in gadoid fish during frozen storage (Castell et al., 1973). Norwegian studies with low temperature (LT) fish meals have indicated that raw material freshness is the most important factor affecting quality. Raw material with a TVN level of less than 50 but up to 90 mg per 100 g fish as assessed by the MgO direct distillation method, is considered necessary for the production of high quality fish meal (Pike et al., 1990).

TMA is found in very low levels in fresh marine fish. This volatile amine, however, can accumulate to high levels during spoilage, mainly as a consequence of the bacterial (*Alteromonus* sp.) reduction of trimethylamine oxide (TMAO) by the enzyme triamine oxidase (Hobbs, 1987) and, to a lesser extent, by other chemical pathways (endogenous fish enzymes, heating, metal ions) (Castell, 1970). Bacteria can also produce TMA from choline and betaine (Dyer and Wood, 1947).

Various methods have been proposed for determining TVN and TMA levels in unprocessed fresh herring and other fish species. These include direct distillation (Halland and Njaa, 1988), steam distillation (Malle and Poumeyrol, 1989), microdiffusion (Aksnes, 1989), colorimetric assay (i.e. picrate method, Auto analyzer) (Dyer, 1945; Ruiter and Weseman, 1976), enzymatic action (Wong et al., 1988), electrodes (Ohashi et al., 1991), gas chromatography (GC) (Perez-Martin et al., 1987) and high performance liquid chromatography (Gill and Thompson, 1984; Fiddler et al., 1991).

Both TVN and TMA values have been reported to vary markedly, depending on the method used (Botta et al., 1984; Connell 1990). In Norway, the currently preferred method for determining TVN is MgO direct distillation, while in Japan microdiffusion is preferred. Thus, the following study was undertaken to compare the various methods commonly in use for determining the levels of TVN and TMA during fish spoilage. The test material used was previously frozen whole ripe male Pacific herring destined for fish meal production.

MATERIALS AND METHODS

RAW MATERIALS

Pacific herring from the commercial fishery were stored in refrigerated sea water until sexed by machine. The mature ripe males were placed in polyethylene bags and snap frozen at -40°C for 24 hours, then stored at -20°C for 90d. Thereafter, fifty fish were selected at random, placed in a polyethylene bag, sealed and stored at 2-5°C for the duration of the experiment. The spoilage of the herring over a 15-day period was determined by daily monitoring of the levels of TVN using each of four methods and TMA using each of three methods.

CHEMICAL ANALYSES

Samples, each comprised of 3 fish, were taken before storage at 2-5°C, and each day (except days 2 and 3) during the 15 days of storage. The partially thawed samples were passed twice through a meat grinder affixed with a plate containing 4.8 mm diameter holes. Each sample was mixed thoroughly and stored at -40°C and analyzed as soon as possible. TVN and TMA levels were determined on each sample in triplicate using the following methods; (a) TVN by direct distillation with MgO using a Kjeldahl distillation apparatus and titration as described by Woyewoda et al. (1986), (b) TVN and TMA by microdiffusion with either K_2CO_3 or KOH. Sample preparation entailed homogenization of 50 gm of fish (Polytron-60 sec.) in 150 ml 7.5% trichloroacetic acid solution. The homogenate was filtered through Whatman #4 filter paper and stored at 4°C until required. Microdiffusion was carried out in a Conway chamber (Obrink, 1955) with titration as described by Murray and Gibson (1972). Chambers were cleaned as recommended by Conway (1957). For TMA analysis, 0.5 ml of neutralized 40% formaldehyde were added to the outer chamber of the dish to block the release of ammonia and primary and secondary amines prior to incubation at 37°C for 2 hours, (c) TVN and TMA by steam distillation with NaOH. The method proposed by the Codex Alimentarius Committee in 1968 as modified by Malle and Poumeyrol (1989) was used. Sample preparation was the same as that

used for the microdiffusion method. Distillation was performed as described by Antonacopoulos and Vyncke (1989). To determine the TMA content, 20 ml of formaldehyde were added to the sample as recommended by Malle and Poumeyrol (1989).

STATISTICAL ANALYSES

The TVN and TMA results were subjected to a one-way analysis of variance, with method as the main effect. Means were compared with Bonferroni's test, (SYSTAT, 1990) with significance level set at $P<0.05$. Correlation coefficients were determined on data obtained with the various TVN and TMA methods over the storage time.

RESULTS AND DISCUSSION

TOTAL VOLATILE NITROGEN

The TVN values reported in this paper were slightly higher than those reported for herring stored on ice (Hjorth-Hansen and Bakken, 1947) and for herring stored anaerobically at 2°C (Halland and Njaa, 1988). The TVN level increased during storage, as monitored by all methods, especially after 8-9 days (Fig. 1). The sudden increase in TVN presumably resulted from an increase in bacterial numbers, since a high correlation between TVN level and bacterial numbers has been reported previously (Reay and Shewan, 1949). The pattern of increase varied little between analysis methods (correlation coefficients of 0.985-0.994). The values for TVN after 15 days of storage ranged from 80 to 114 mg N/100g fish (as is basis), depending upon the method employed. The consistently higher TVN values observed with direct distillation with MgO than with the two microdiffusion methods may have occurred as a result of protein breakdown during atmospheric distillation (Pearson and Muslemuddin, 1968; 1969; Botta et al., 1984).

The results demonstrated that TVN is not a good indicator of initial spoilage but is a good indicator of advanced spoilage. This finding agrees with those of earlier studies (Hjorth-Hansen and Bakken, 1947; Reay and Shewan, 1949).

TRIMETHYLAMINE

The levels of TMA paralleled those of TVN during storage of herring at 2-5°C (Fig. 1). Only after 8 days was a significant amount of TMA present. These results indicated that the differences between the methods were reasonably constant (correlation coefficients of .890-.979).

In this experiment interference from DMA should not have been a problem, since it has been shown by Reay and Shewan (1949) and Fernandez-Salguero and Mackie (1987) that, during spoilage of herring, DMA is produced only in small amounts (< 1.0 mg N/100g).

In the present study the lag period for TMA production was longer (9 days) than the lag period of 5 days reported by Fernandez-Salguero and Mackie (1987) for whole herring stored at 5°C. The lower temperatures of 2-5°C for the Pacific herring stored in this experiment could account for the lower TMA levels, since the production of TMA in herring is temperature dependent (Sigurdson 1947). However, the TMA levels in the present study are similar to those noted for whole herring stored on ice (Damoglou, 1980; Fernandez-Salquero and Mackie, 1987) and in refrigerated sea water (Smith et al., 1980). This can be explained by the fact that the freezing of fish below -6°C reduces the bacterial population by 60-90%, but upon thawing and storage on ice, the bacterial numbers (*Pseudomonas-Alteromonas* and *Moraxella-Acinetobacter* groups) recover quickly and spoilage progresses at a rate similar to that observed for fish on ice that have not been frozen (Hobbs, 1987).

The lag phase for bacterial growth in cod has been found to be increased by 2-5 days as a result of previous frozen storage for 3 to 6 months. Experiments conducted at the Torry Research Station showed that freezing may also increase the lag phase for TVN and TMA levels (Shewan, 1961). Luijpen (1958) found that TMA and ammonia formation were suppressed for up to 5 days in previously frozen cod relative to non-frozen cod stored at 2°C.

These results support previous findings indicating that neither TVN nor TMA are good indicators of early quality changes but are of value for monitoring advanced spoilage (Sigurdsson, 1947; Reay and Shewan, 1949; Smith et al., 1980). Although there are differences between methods regarding TVN and TMA values, all seven methods resulted in similar trends and each appears to be suitable for use as an index of advanced spoilage.

The low cost of the microdiffusion method (Botta et al. 1984) and its simplicity and speed of use without loss of precision would make microdiffusion the method of choice for TVN and TMA measurement.

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Figure 1. Content of total volatile nitrogen (TVN) determined by four methods and trimethylamine (TMA) determined by three methods in previously frozen Pacific herring stored at 2-5°C for 15 days. Values are on an "as is" basis.