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Quantitative Determination of Dimethyl- and Trimethylamine in Fish Protein Concentrate

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Dimethylamine (DMA), 25 to 150 ppm, and trimethylamine (TMA), 5 to 10 ppm, were detected in samples of fish protein concentrate (FPC) prepared from frozen red hake (*Urophycis chuss*) and Pacific hake (*Merluccius productus*) by isopropyl alcohol extraction. The following compounds were also identified by combined gas–liquid chromatography and mass spectrometry: methyl mercaptan, acetaldehyde, propionaldehyde, methylene chloride, acetone, chloroform, isopropyl alcohol, ethyl alcohol, butanone, toluene, dimethyl sulfide, and dimethyl disulfide. The probable presence of ethylamine and a butylamine was indicated.

The occurrence of secondary amines (Kröller, 1950; Lerenkov *et al.*, 1960; Miyahara, 1960; Preusser, 1966; Wick *et al.*, 1967) and the presence of nitrite (Hanni, 1953; Kamm *et al.*, 1965; Phillips, 1968) in various foods are well established. Significant levels of dimethylamine (DMA), rather than trimethylamine (TMA), are formed in several species of hake currently utilized in the preparation of fish protein concentrate (FPC). Since some dialkylnitrosamines are potent carcinogens (Afkham *et al.*, 1967; Magee and Barnes, 1967), the presence of DMA in FPC prepared from hake may have significant toxicological implications. In the presence of nitrite, DMA can be nitrosated under acidic conditions to form dimethylnitrosamine (DMN). The formation of DMN has been demonstrated in herring meal (Ender *et al.*, 1964; Sakshaug *et al.*, 1965) and in smoke-processed marine fish such as sable, salmon, and shad (Fazio *et al.*, 1971). Although DMN, and possibly other nitrosamines, could be formed in the gastric contents during digestion (Sander, 1967; Sander *et al.*, 1968; Sen *et al.*, 1969), it should be emphasized that the lowest level of DMN which could elicit a carcinogenic response has not, at present, been established.

Wick *et al.* (1967) identified DMA, TMA, and several other volatile amines in FPC prepared from red hake (*Urophycis chuss*); however, no quantitative data were presented. This investigation was initiated to quantitatively determine the DMA and TMA contents of FPC prepared from red hake (*U. chuss*) and Pacific hake (*Merluccius productus*).

EXPERIMENTAL SECTION

Sample Preparation. Regular fish protein concentrate (R-FPC), prepared from frozen red hake (*Urophycis chuss*) by extraction with isopropyl alcohol, was obtained from the National Center for FPC, National Marine Fisheries Service, College Park, Md. In attempts to increase protein functionality (Anglemier and Petropakis, 1972), FPC was modified essentially as follows. R-FPC was hydrolyzed with 0.2 *N* NaOH at 95–100° for 14 min in a closed system. The mixture was centrifuged and the protein in the supernatant fraction was precipitated at pH 4.5 with 6 *N* HCl. The resultant precipitate, redissolved and adjusted to pH 7.0 with NH_4OH, was then spray dried. HmPh-FPC was prepared from the supernatant remaining after isoelectric precipitation at pH 4.5 by treatment with sodium hexametaphosphate.

Gas–Liquid Chromatography. A stainless steel column (5.5 m × 3 mm o.d.), containing 40–60 mesh Graphon (Cabot Corp., Billerica, Mass.) coated with 2% tetraethylenepentamine (TEP), was used in conjunction with an alkali flame ionization detector (AFID) for the selective separation and quantitative determination of DMA and TMA. Approximately 30 ml of 10% NaOH was added to each FPC sample (5 g) contained in a screw-capped bottle (250 ml), and the amine contents were determined by equilibrium vapor analysis after heating at 60° for 25–30 min (Miller *et al.*, 1972).

A column (3.7 m × 3 mm o.d.), containing acid/base washed Celite 545 (60–80 mesh) coated with 20% 1,2,3-*tris*(2-cyanoethoxy)propane (TCEP), was used with a gas entrainment, on-column trapping procedure (Morgan and Day, 1965) for the identification of relatively low-boiling compounds. Approximately 25 ml of double, glass-distilled water was added to each 5-g portion of FPC contained in screw-capped bottles (250 ml). Each sample, containing a few milligrams of 1-tetradecanol to control foaming, was tempered at 60° for 30 min and the volatiles were condensed on the column packing using a nitrogen gas purge rate of 12 ml/min for 10 min. The nitrogen purge time was increased when more concentrated samples were needed for mass spectral analysis.

The TCEP and Graphon plus TEP columns, installed in Varian Aerograph series 1200 and 1400 gas chromatographs, respectively, were operated isothermally at 60° with a nitrogen gas flow rate of 30 ml/min. The detector and injector port temperatures were 210° and 190°, respectively. The series 1400 instrument was equipped with an AFID and the series 1200 instrument had a flame ionization detector (FID).

Mass Spectral Analysis. An F&M Model 810 gas chromatograph was used in conjunction with an Atlas CH-4

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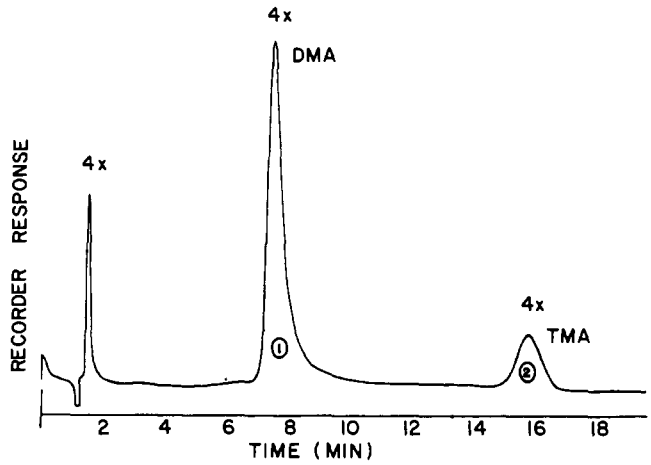

Figure 1. AFID and recorder response to volatile components from R-FPC. Column: Graphon and TEP (2%), 5.5 m × 3 mm o.d.

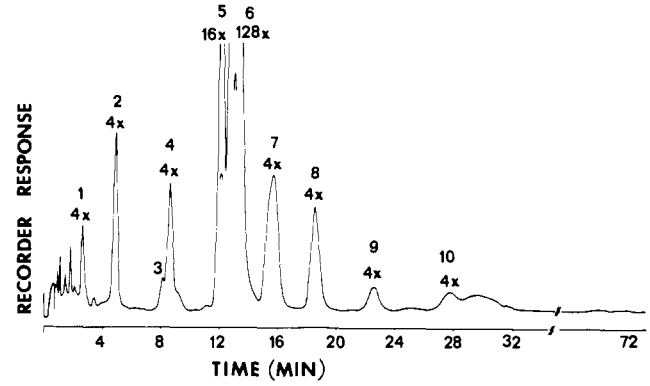

Figure 2. FID and recorder response to volatile compounds from an aqueous slurry of FPC. Column: TCEP (20%), 3.7 m × 3 mm o.d.

mass spectrometer for all mass spectral analyses as previously described (Miller *et al.*, 1972). Spectra were scanned from m/e 12 to m/e 250 in 2.5 sec.

RESULTS AND DISCUSSION

Peak heights obtained by equilibrium vapor analysis for various concentrations of TMA and DMA added to FPC were plotted and standard curves were constructed. As previously reported (Miller *et al.*, 1972), TMA (10 ppm) and DMA (50 ppm) were easily detected and the linearities of response were highly reproducible. The lower limits of detection for DMA and TMA were approximately 25 and 5 ppm, respectively.

The AFID and recorder response to volatile components from R-FPC is illustrated in Figure 1. Peaks 1 and 2, DMA (150 ppm) and TMA (5 ppm), respectively, were confirmed by retention times of authentic compounds, peak enhancement, and comparison of mass spectra with known spectra. The mean values and corresponding standard deviations for several determinations of DMA and TMA in R-FPC were 151.33 ± 3.21 and 5.06 ± 0.61 ppm, respectively. The peak with a retention time at approximately 2 min is ammonia.

Two additional FPC samples, obtained from the National Center for FPC at College Park, Md., contained approximately 25 ppm of DMA and 5 ppm of TMA. Modified FPC (M-FPC) and HmPh-FPC contained approximately 25 ppm of DMA and slightly less than 5 ppm of TMA. Although isoelectric precipitation at pH 4.5 and sodium hexametaphosphate treatment reduced the initial level of DMA sixfold, the concentration of TMA was not altered appreciably. A FPC sample prepared from Pacific hake (*M. productus*) and obtained from the National Marine Fisheries Service FPC demonstration plant at Aberdeen, Wash., contained 25 ppm of DMA and 10 ppm of TMA. Although most of the DMA and TMA values reported herein are very close to the lower limits of detection by headspace analysis, the results of duplicate determinations were highly reproducible.

With the exception of R-FPC, the DMA values reported above are similar to the DMA content of an FPC sample previously analyzed by Wick *et al.* (1967). According to Mirvish (1970), this sample contained 152 mg/kg of mixed amine hydrochlorides of which 25%, or approximately 38 ppm, was estimated to be DMA·HCl.

The FID and recorder response to volatile compounds from an aqueous slurry of FPC is shown in Figure 2. Compounds identified are listed as follows with respective peak numbers: (1) methyl mercaptan, (2) acetaldehyde, (3) propionaldehyde, (4) methylene chloride, (5) acetone and chloroform, (6) isopropyl alcohol and ethyl alcohol, (7) not identified, (8) butanone, (9) toluene, and (10) dimethyl disulfide. Chloroform and ethyl alcohol were minor constituents of peaks 5 and 6, respectively. The mass spectrum for peak 7, comprised of two components, indicated major ions at m/e 57 and 58 and minor ions at m/e 71 and 86; however, the relative intensities observed could not be correlated with known spectra. Similar chromatographic patterns were observed for all FPC samples examined. Several of the above components, such as methylene chloride, chloroform, and toluene, might be the result of impurities in the solvent or atmospheric contaminants that accumulated during the preparation and storage of the FPC. Dimethyl sulfide was also detected and identified in alkaline slurries of several FPC samples. Wick *et al.* (1967) were not able to detect sulfur-containing compounds under their experimental conditions.

Minor components, with retention times similar to those of ethylamine (EA) and a butylamine (BA), were also detected in several of the FPC samples analyzed. Wick *et al.* (1967) tentatively identified EA and a BA, as well as other amines, in FPC prepared from red hake (*U. chuss*) by isopropyl alcohol extraction.

Although several volatile compounds were identified in the FPC samples prepared from frozen hake, the presence of DMA is of particular importance when one considers the possibility of DMN formation. Any potential risk to human health arising from the formation of nitrosamines certainly deserves special attention and requires further investigation. Since species of hake characteristically form DMA during frozen storage (Castell *et al.*, 1971; Miller *et al.*, 1972), the fresh product or other species of fish (*e.g.*, menhaden), which contains lower levels of DMA (Spinelli, 1972), should be considered for utilization in the manufacture of FPC.

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Hypoxanthine Measurement in Assessing Freshness of Chilled Channel Catfish (*Ictalurus punctatus*)

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Existing methods were modified for the inexpensive, objective measurement of hypoxanthine in muscle of channel catfish (*Ictalurus punctatus*) stored at 2° *postmortem* for up to 22 days. Sensory ratings were performed for appearance, color, aroma, texture, and flavor and compared to the rate of hypoxanthine accumulation in the same fish. Measurement of the purine, especially prior to noticeable spoilage, shows potential value as an index in assessing freshness of chill-stored catfish.

Nucleotide degradation in fish muscle commences at death or early *postmortem* and proceeds throughout storage. The autolytic processes involve dephosphorylation and deamination of adenosine triphosphate to form inosine monophosphate, which is degraded to inosine and finally to hypoxanthine. Examination of nucleotide degradation in fish muscle as a means for assessing fish freshness and quality has recently received considerable attention (Hiltz *et al.*, 1971). Measurement of the accumulation of hypoxanthine in particular has shown potential as an index of freshness, especially in marine fishes (Burt *et al.*, 1969; Fraser *et al.*, 1968a,b; Jones *et al.*, 1964; Kassemsarn *et al.*, 1963; Spinelli, 1967). Although information on hypoxanthine formation in freshwater fishes is less abundant, its increased levels during storage have been demonstrated (Dugal, 1967; Kuusi and Aalto, 1968) and its usefulness in assessing freshness has been noted (Bligh, 1971).

Methods of culturing, harvesting, and processing presently employed in the channel catfish (*Ictalurus punctatus*) industry are varied and inconsistent. Commercially available catfish may have been fed one of many diets and cultured in ponds, raceways, or in the wild; they may or may not have been iced at their point of harvest and transported long distances, or they may have been harvested, slaughtered and chilled, iced, or frozen all within a period of minutes. These variations in handling may result in catfish of different body composition and microflora. In any case, the body temperature at harvest can range from 20 to 24°, which demands an immediate reduction in order to combat bacterial growth and autolytic breakdown. It would be highly desirable to rely on a simple, rapid, and preferably inexpensive test to assess the quality of channel catfish as they are received at the processing plant or as they are presented in the market place. Such a test should not be greatly influenced by variation in environmental conditions to which the fish were exposed before death.

The present study involves the measurement of hypoxanthine in stored chilled channel catfish by a procedure derived from previously existing methods (Burt *et al.*, 1968, 1969; Jones *et al.*, 1965). The procedure appears to be valuable in assessing freshness of chilled channel catfish.

EXPERIMENTAL SECTION

Materials. Pond-raised channel catfish averaging 400 g (live weight) were obtained locally and transported in an aerated tank to the Food Science Laboratory, Experiment, Ga., where they were stunned with alternating current, decapitated, eviscerated, and skinned. Fish were then washed, packaged individually in polyethylene bags, and stored at 2° in a constant temperature cabinet until analyses were performed.

Organoleptic Evaluation. Four catfish were removed from storage at 2° after 0, 2, 4, 7, 9, 11, 14, 17, and 22 days. A fillet was removed from one side of each fish and retained for hypoxanthine analysis; the remaining portions of the fish were subjected to sensory evaluation by a trained six-member panel. After ratings were assigned to the raw fish for appearance, color, and aroma, the fish were baked at 350°F for 25 min in sealed aluminum foil pouches and ratings were performed for texture and flavor. A score of 9 on a 9-point hedonic scale indicated extremely good, while a 1 indicated extremely poor quality for the particular organoleptic character being judged.

Reagents for Hypoxanthine Determination. The reagents required are among those described in an earlier publication (Burt *et al.*, 1969) from which this procedure has been adapted. The procedure followed here requires 0.6 *N* perchloric acid, buffered potassium hydroxide [prepared by combining 20% KOH (w/v) with 0.4 *M* potassium phosphate buffer at pH 7.6 in the ratio of 42:56 (v/v)], 0.17 *M* phosphate buffer (pH 7.6) containing 20 μg of 2,6-dichlorophenolindophenol per ml, xanthine oxidase (General Biochemicals, Chagrin Falls, Ohio, I.U.B.

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