Decomposition of Trimethylamine Oxide and Changes in Protein Extractability during Frozen Storage of Minced and Intact Hake (*Merluccius productus*) Muscle

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During frozen storage at −20°C, dimethylamine (DMA) and formaldehyde (FA) were produced in hake fillets. Formation of DMA and FA was greatly accelerated in minced muscle. Trimethylamine (TMA), DMA, and FA determined immediately after mincing of the muscle were 2–4 times greater than determined in the intact fillet. This corresponded to a much lower trimethylamine oxide content in minced muscle. The formation of DMA and FA was not altered by exclusion of oxygen. Dimethylamine and FA formation in intact fillets packaged in vacuum- or air-sealed moisture vapor-proof pouches or wrapped in polyethylene followed the same pattern during storage. Dimethylamine and FA formation increased steadily during storage in minced muscle packaged in the same manner as the intact fillet. After 4 months the content of DMA and FA in minced muscle was 1.5–2 times greater than in the intact fillet. The rapid decrease in the amount of total extractable protein corresponded to the increase in FA and DMA content.

The determination of trimethylamine (TMA) as an indicator of freshness (actually of decay) has been a useful criterion for evaluating the quality of fish (Castell, 1970). Formation of TMA in fish muscle has been linked to the action of bacterial enzymes, since marine microorganisms can convert trimethylamine oxide (TMAO) to TMA (Budd and Spencer, 1968) and a close relationship exists between the numbers of bacteria in the muscle and the amount of TMA that is formed (Laycock and Regier, 1971).

Recently, Harada and Yamada (1971) and Amano and Yamada (1965) have isolated an enzyme system from fish and shellfish that reduces TMAO to TMA, dimethylamine (DMA), and formaldehyde (FA). Although these experiments were carried out at 0°C and above, there is evidence to indicate that a similar reaction occurs in some fish during frozen storage (Castell *et al.*, 1971; Tokunaga, 1970). Furthermore, Mendenhall (1969) found that the concentration of FA in ground whole hake reached antimicrobial levels during frozen storage.

This study was initiated to follow the formation of FA and degradation of TMAO in intact and minced muscle of Pacific hake during frozen storage. Further studies followed the changes in muscle protein during frozen storage and to see whether or not these changes were affected by different packaging methods.

EXPERIMENTAL TECHNIQUES

Pacific hake, locally caught, were randomly divided into two groups. One group was washed, filleted, packed into stainless steel trays, and frozen at −30°C. The other group was washed, dressed, and fed through a Yanagiya Fish Separator (Yanagiya Machinery Works, Ltd., Yamaguchi Pref., Japan). Separation of muscle from the skin and bone was carried out at room temperature. The temperature of the fillets and minced muscle never exceeded 10°C before freezing. Initial samples of hake muscle were analyzed within 15 min after filleting and mincing. The minced muscle was packed in stainless steel trays and frozen at −30°C. After 16 hr the blocks were removed from the freezer and sawed into 0.5-in. × 3.5-in. × 3.75-in. portions. Portions were immediately air- or vacuum-sealed in moisture vapor-proof pouches or wrapped in polyethylene. The hake portions were held at −20°C.

Analysis. Two portions (300 g) of frozen muscle samples prior to each extraction were ground twice in a meat chopper and mixed well. For determination of soluble protein content, muscle-extractant homogenates were prepared using techniques previously described by Ravesi and Anderson (1969). Extractants for water-soluble or sarcoplasmic protein (0.05 μ phosphate buffer; 0.0157 *M* Na_2HPO_4, 0.0031 *M* KH_2PO_4, pH 7.5) and total extractable protein (0.86 *M* NaCl, 0.02 *M* $NaHCO_3$, pH 7.2) were prepared by blending homogenates in a Virtis Model 45 homogenizer at high speed for 4 min. Seven grams of ground muscle were blended with 273 ml of extraction solution and a portion of the homogenate was centrifuged at 1000 × *g* for 20 min. Protein content of the centrifuged solutions was determined by the biuret method (Snow, 1950). A factor of 6.25 was used to convert nitrogen to protein content using egg albumin as the standard. Results were expressed as percent protein extractable, the total protein content of the muscle having been determined at each sampling by a semimicro-Kjeldahl method.

Ten grams of ground muscle were blended with 40 ml of 5% TCA (trichloroacetic acid) and the supernatant was used for the determination of TMAO, TMA, DMA, and FA. The method of Yamagata *et al.* (1969) for reducing TMAO to TMA was used to determine TMAO. The picric acid procedure of Dyer (1945) was used to measure TMA. The difference between total TMA (TMAO + TMA) and TMA values represents the amount of TMAO. Dimethylamine was measured by the copper–dithiocarbamate method of Dyer and Mounsey (1945). Dimethylamine will react in the picric acid determination for TMA, giving 21% of the color as an equivalent concentration of TMA (Dyer, 1945). Since the interference of DMA was found to be constant over a wide concentration range (0–20 μg/ml), the presence of DMA in the TMA–picrate test can be determined. The TMA and TMAO values reported in the figures have been corrected for

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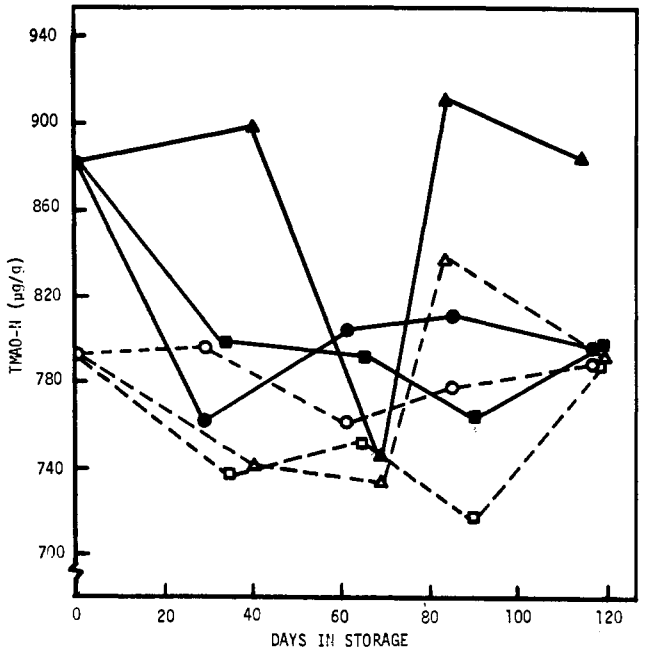

Figure 1. Change in TMAO–N in hake flesh at −20°C. ●—●, intact, vacuum-sealed; ■—■, intact, air-sealed; ▲—▲, intact, polyethylene wrap; ○- - -○, minced, vacuum-sealed; □- - -□, minced, air-sealed; △- - -△, minced, polyethylene wrap

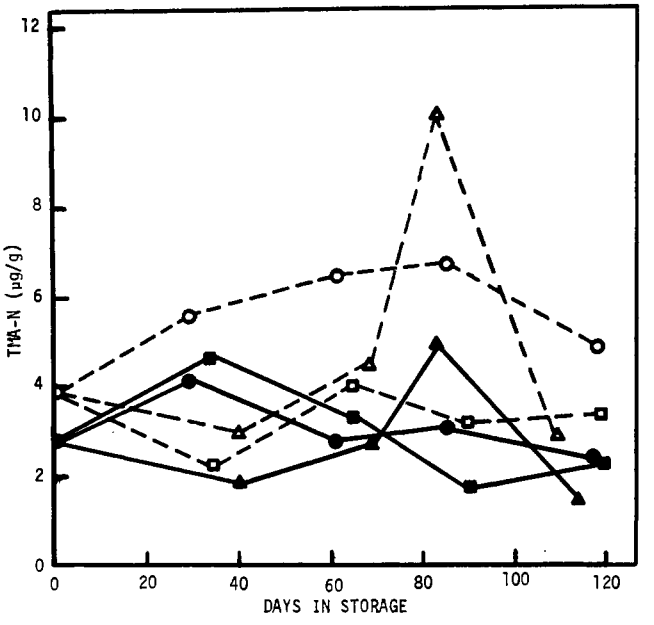

Figure 2. Change in TMA–N in hake flesh at −20°C. (See Figure 1 for symbol definitions)

the presence of DMA and are expressed as nitrogen content (Dyer, 1959; Dyer and Mounsey, 1945).

Formaldehyde was determined by the method of Sawicki *et al.* (1961). Five milliliters of 0.4% aqueous solution of 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate were mixed well with 1 ml of supernatant in a 100-ml volumetric flask and held for 25 min in a water bath at 40°C. Then, 20 ml of 0.2% $FeCl_3$ in 1 *N* HCl was added with mixing and left for an additional 25 min. The solution was diluted to 100 ml with distilled water and the absorbance determined at 670 nm against a blank. Standard solutions were prepared with an analytical reagent grade formaldehyde solution.

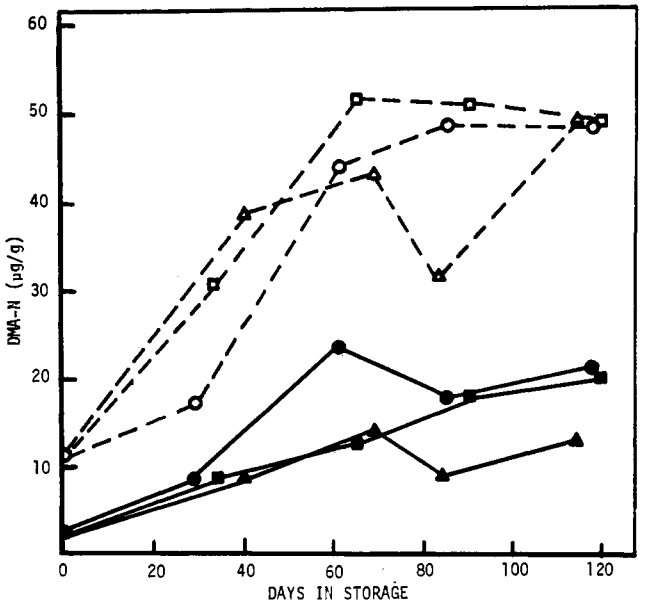

Figure 3. Change in DMA–N in hake flesh at −20°C. (See Figure 1 for symbol definitions)

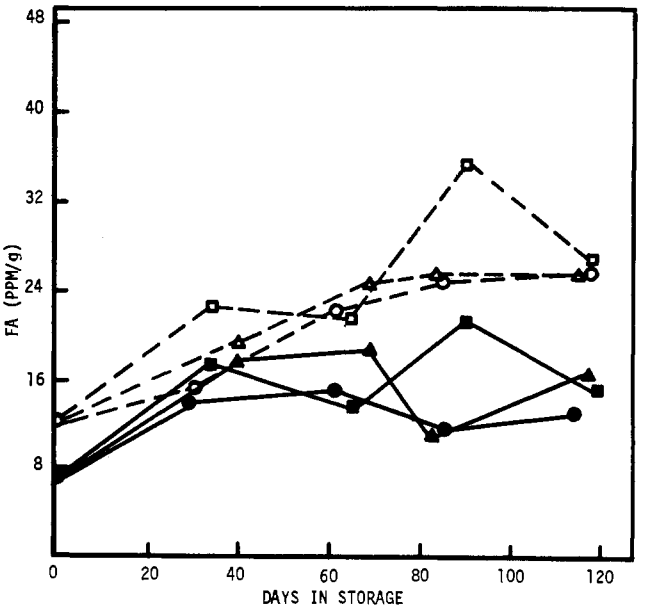

Figure 4. Change in FA in hake flesh at −20°C. (See Figure 1 for symbol definitions)

The points in Figures 1–6 represent the mean value of duplicate samples.

RESULTS AND DISCUSSION

Mincing of hake muscle accelerated the reduction of TMAO (Figure 1). This was accompanied by an increase in TMA, DMA, and FA (Figures 2–4). The changes in TMA and DMA content of hake fillets were similar to changes in cod fillets reported by Castell *et al.* (1970). The changes in TMA of both minced and intact hake muscle remained fairly constant during storage. However, the content of DMA in intact fillets and particularly in minced muscle rose continuously during storage. The FA content of minced and intact hake muscle also increased steadily during frozen storage (Figure 4).

Tokunaga (1970) found that the concentration of TMAO, TMA, and DMA varied greatly between light and dark

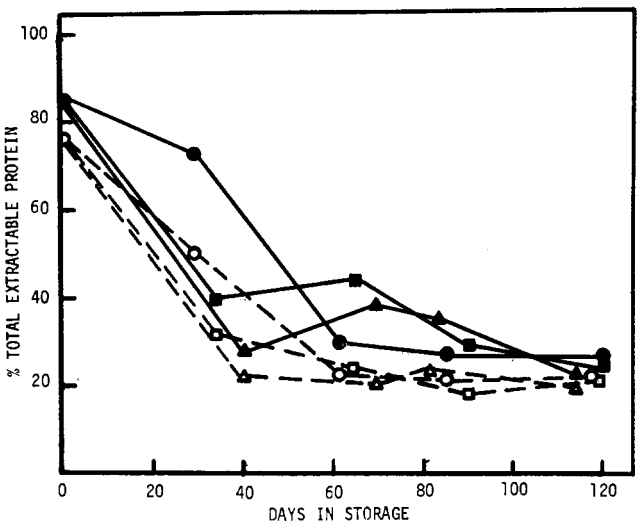

Figure 5. Change in total extractable protein in hake flesh at −20 °C. (See Figure 1 for symbol definitions)

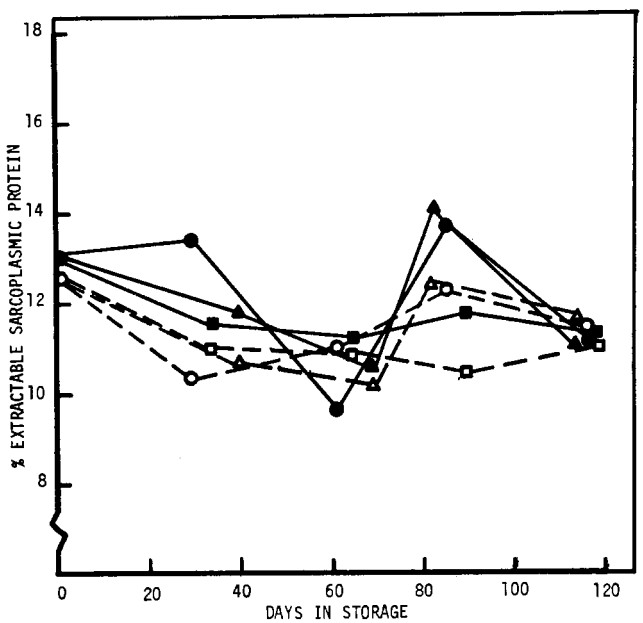

Figure 6. Change in sarcoplasmic protein in hake flesh at −20 °C. (See Figure 1 for symbol definitions)

muscles of fish. The sharp drop in TMAO content of intact fillets at 70 days of frozen storage (Figure 1) may be due to a small amount of dark muscle included in the sample of frozen fish portions. This may also explain the large concentration of TMA found in minced muscle at 82 days of frozen storage (Figure 2). Furthermore, the presence of dark muscle in the samples may play a significant role in the reduction of TMAO since it has recently been reported that the removal of dark muscle from hake fillets markedly reduces DMA formation during frozen storage (Castell *et al.*, 1971).

Packaging seemed to have little effect on the breakdown of TMAO and the formation of TMA and DMA. After 115 days of storage at −20°C, changes in DMA content of fillets vacuum- or air-sealed in moisture vapor-proof pouches and polyethylene wrap were very similar. The DMA content of minced muscle increased rapidly and reached a higher level than the intact fillet handled in an identical manner (Figure 3).

The alteration of muscle protein as measured by the decrease in total extractable protein (Figure 5) was retarded in the vacuum-packaged portions. The extractable protein was higher in both the vacuum-packed portions of minced muscle and intact fillets after 30 days of storage than in similar minced muscle and fillets packaged in air-sealed pouches or polyethylene wrap. The content of FA in the vaccum-packed minced and intact muscle was correspondingly lower. It is difficult to speculate if a relationship exists between the concentration of FA in the intact fillet and the extractability of the myofibrillar protein. However, the large decrease in total extractable protein in the minced muscle after 60 days seems to reflect the higher concentrations of FA found in the different minced muscle samples.

There was a slight decrease in extractable sarcoplasmic protein during frozen storage (Figure 6). Apparently, the "denaturation" of fish muscle proteins during frozen storage is mainly due to the alteration of myofibrillar protein.

The determination of DMA, rather than TMA, may be a useful method for evaluating the changes in quality of hake and hake-like species of fish during frozen storage. The increase in DMA may not only be an index of the formation of FA, but also the interaction of FA with myofibrillar protein. The FA formed during the reduction of TMAO to DMA may be rapidly interacting with the myofibrillar protein in a manner similar to the cross-linking with bovine fibrinogen described by Mihalyi (1963). The interaction of FA with myofibrillar protein could explain, in part, the undesirable textural changes that occur during the frozen storage of Pacific hake.

Another system that DMA may be monitoring is the denaturation of protein due to a lipid–protein complex (Anderson and Ravesi, 1969). In the reduction of TMAO to DMA, free fatty acids present in the flesh may become partially oxidized. These oxidized fatty acids as well as FA could complex with the contractile protein, resulting in the deterioration of muscle.

ACKNOWLEDGMENT

The authors thank Lewis L. Richardson for his technical assistance.

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Received for review March 17, 1972. Accepted May 17, 1972. Technical Paper No. 3288, Oregon Agricultural Experiment Station, Corvallis, Oregon. Paper presented at the 162nd National ACS Meeting, Washington, D.C. Investigation supported in part by the National Oceanic and Atmospheric Administration (maintained by the U.S. Department of Commerce) Institutional Sea Grant 2-35187.