The Possible Nitrosation of Amines in Smoked Chub

Studies were undertaken on the formation of dimethylnitrosamine (dmna) in aqueous model systems containing methyl amines and sodium nitrite. The reaction conditions (pH, reaction time, and temperature) were more severe than those occurring during the commercial processing of nitrite treated smoked chub. Polarographic analyses indicated that detectable amounts of dmna (1.4×10^{-8} mole) were not produced. It was concluded that in all probability dmna does not form in concentrations greater than 10 ppb during the smoking process.

Possible formation of the carcinogen dimethylnitrosamine (dmna) in food products has aroused wide concern (Lijinsky and Epstein, 1970; The Lancet, 1968). Accordingly, the issuance of a regulation by the U.S. Food and Drug Administration on the safe use of sodium nitrite in preserving smoked chub from the Great Lakes required evidence that dmna does not form via the nitrosation of methyl amines. A petition for a commercial process included 100 to 200 ppm of residual sodium nitrite in loin muscle and a minimum temperature of 160° F (71° C) for at least 30 min. A regulation for this process has been recently published (Federal Register, 1969).

In analyses of amines in chub, maximum concentrations of 25 ppm of trimethylamine (tma) and 107 ppm of trimethylamine oxide (tmao) have been documented (Grieg, 1970). A concentration of 2 ppm of dimethylamine (dma) has also been reported (Grieg, 1970). The pH values of smoked chub varied from 5.9 to 7.3 (unpublished data, Bureau of Commercial Fisheries, Ann Arbor, Mich., 1966), with the majority of the values in the range of 6.4 to 6.8 (Weckel and Wosje, 1966). It seemed probable from the studies of Sander *et al.* (1968), that the pH values were too high and the amine (tma, tmao, and dma) concentrations too low for the nitrosation reaction to take place. To test this hypothesis we studied in aqueous model systems the reaction under more severe conditions (pH, reaction time, temperature, and amine concentrations) than would occur during the commercial process.

Solutions (50 ml) were buffered at pH 6.4 and 5.8 with phosphate-citrate acid (Colowick and Kaplan, 1955). The concentrations of amines were varied from 200 to 2000 ppm (Table I). Reactions were conducted for 2.5 hr at 80 and 100° C in solutions containing 400 ppm of sodium nitrite (this concentration represents an estimate of the maximum sodium nitrite level reached during processing). Reaction products were analyzed for dmna by conventional DC polarography using a Heath EUW 401 operational amplifier, chopper stabilizer, and a three-electrode polarograph (Schlumberger Products Corp., St. Joseph, Mich.) and the electrolyte solutions and workup procedures described by Heath and Jarvis (1955). A fast scan system consisting of Chemtrix modules (Chemtrix Corp., Beaverton, Ore.) was also employed. The lower limit of detection was 1 μg (1.4×10^{-8} mole) of dmna.

Dmna was not detected in any reaction conducted at 80° C under the conditions shown in Table I. About 1 μg of dmna was detected at a tma concentration of 400 ppm at 100° C and a pH of 6.4. However, a slightly lower pH (5.8) resulted in the synthesis of 8.5 μg of dmna. These results suggest that a slight increase in the acidity of the chub may result in a significant increase in the nitrosation of the methyl amines (Sander *et al.*, 1968). Under all conditions shown in Table I,

Table I. Reactions of Amines with $NaNO_2$ in Aqueous Solutions

Amine mole × 10^{-4}	Concentration ppm	Mole Ratio amine:nitrite	pH	Dmna Formed (μg) 80° C	100° C
Tma					
0.85	100	0.29	6.4	...	N.D.
0.85	100	0.29	5.8	N.D.[a]	...
1.7	200	0.58	6.4	N.D.	N.D.
1.7	200	0.58	5.8	N.D.	...
3.4	400	1.2	5.8	...	8.5
3.4	400	1.2	6.4	...	1.0
5.1	600	1.8	6.4	...	2.1
6.8	800	2.3	6.4	N.D.	2.6
8.5	1000	2.9	6.4	...	4.1
10.2	1200	3.5	6.4	N.D.	5.7
13.5	1600	4.7	6.4	N.D.	5.7
16.9	2000	5.8	6.4	N.D.	6.9
Tmao					
5.3	800	1.8	6.4	N.D.	N.D.
6.7	1000	2.3	5.8	...	N.D.
8.0	1200	2.8	6.4	N.D.	N.D.
10.6	1600	3.7	6.4	N.D.	N.D.
13.3	2000	4.6	6.4	N.D.	N.D.
Dma					
8.9	800	3.1	6.4	N.D.	N.D.
11.1	1000	3.8	5.8	...	N.D.
13.3	1200	4.6	6.4	N.D.	N.D.
17.7	1600	6.1	6.4	N.D.	N.D.
22.2	2000	7.6	6.4	N.D.	N.D.

Constants: 400 ppm (2.9×10^{-4} mole) $NaNO_2$; 2.5 hr reaction time.

[a] N.D.: not detected.

dma and tmao did not react to yield detectable amounts of dmna.

The results of these studies, conducted under exaggerated reaction conditions, indicate that, in all probability, concentrations of dmna greater than 10 ppb would not result from the commercial processing of chub. It is recognized, however, that the aqueous model systems do not fully reflect the actual chemical environment in the smoked chub. These results are supportive of the data obtained by Howard *et al.* (1970) with smoked chub. These workers, employing glc techniques, were unable to find evidence for the nitrosation of amines under the prescribed processing conditions.

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