Formation of Dimethylnitrosamine from Commercial Lecithin and Its Components in a Model System

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The formation of dimethylnitrosamine (DMNA) from choline chloride has been reported. Choline is commonly found as a component of lecithin. Since lecithin is present in animal tissue and is a widely used food additive, DMNA formation in foods containing both lecithin and nitrite is a possibility. When 4.56 mmol of various natural lecithins and their components was allowed to react with sodium nitrite at pH 5.60 under model system conditions, DMNA was formed in varying concentrations (0.70 to 30.76 ppm).

Recently we reported (Fiddler et al., 1972a) that naturally occurring quaternary ammonium compounds react with sodium nitrite, under mildly acidic conditions, to form dimethylnitrosamine (DMNA). One of the quaternary ammonium compounds tested was choline. This compound, when heated, decomposes to trimethylamine (TMA) which can further demethylate to the dimethylamine (DMA) precursor of the nitroso compound.

Choline occurs naturally in tissue, either free or combined, principally in the form of phospholipid lecithins. This class of compounds, which are fatty acid–choline esters of glycerophosphoric acid, is found in plant and animal tissues. They occur in higher concentrations in organ meats, which are used in the preparation of some comminuted meat products, than in skeletal meats. Lecithin preparations are used commercially as emulsifying agents and are present in sprays used to prevent the sticking of food to pots and pans during cooking. Lecithins are used as dietary supplements and have also been proposed to prevent bacon slices from sticking together (Wrobel and Rendek, 1973).

Asatoor and Simenhoff (1965) found that the source of urinary dimethylamine was either ingested or endogenous choline and lecithin. Recently, Möhler and Hallermayer (1973) reported the formation of DMNA from egg lecithin and nitrite.

In view of the potential carcinogenicity of DMNA to humans, the reaction between commercial lecithin samples and nitrite in a model system under conditions simulating processing of some meat products was examined and the results of this study reported herein.

EXPERIMENTAL SECTION

The lecithins were used as purchased: bovine, egg, soy, vegetable, and synthetic from Nutritional Biochemical Co. and edible soy from Wm. Luddy Company. Phosphorocholine chloride was purchased from Calbiochem, choline chloride from Sigma Chemical Co., and dimethylamine and trimethylamine hydrochlorides from Eastman. In addition, the commercial bovine lecithin (60%) was purified to approximately 95+% using the method described by Levene and Simms (1921). Glycerylphosphorylcholine was prepared from egg lecithin following the method reported by Brockerhoff and Yurkowski (1965). Phosphorocholine chloride was converted from the calcium salt to the free acid by using oxalic acid to precipitate the calcium. The lecithins were extracted with ether; the extracts were concentrated and analyzed by gas–liquid chromatography for free dimethylamine and trimethylamine to determine whether these decomposition products were present prior to reaction with nitrite. A 9 ft × ⅛ in. stainless steel column containing 40–60 mesh 2% TEPA on Carbopack B (Miller et al., 1972) in an F&M Model 810 gas chromatograph was used. The operating conditions were: column temperature, 71°; injection port, 160°; detector, 200°. The flow rates were: helium, 12; air, 160; and hydrogen, 53 ml/min. The limit of sensitivity for both DMA and TMA was less than 1 ppm.

Procedure. Solutions containing 4.56 mmol of dimethylamine, trimethylamine, choline chloride, phosphorocholine chloride, or glycerylphosphorylcholine, or suspensions containing 4.56 mmol of the lecithins (the lecithin calculations were based on elemental phosphorus analysis) were prepared in 50 ml of pH 5.60 buffer (0.5 *M* NaOH, 0.5 *M* KH_2PO_4). To each of these were added 22.8 mmol of sodium nitrite dissolved in 15 ml of H_2O. The mixtures were stirred and heated at 78° (172°F) for 4 hr. This temperature was selected since it is close to the smokehouse temperature used in processing some meat products. The reaction mixtures were cooled and then extracted 3× with 75 ml of methylene chloride. The extracts were combined, dried by passing through anhydrous sodium sulfate into a Kuderna-Danish apparatus, and concentrated to 8 ml on a steam bath. The resulting viscous liquid was subjected to column chromatography using acidified Florisil (10 g of Florisil and 8 ml of 6 *N* HCl), washed with 200 ml of *n*-hexane, and then eluted with 100 ml of methylene chloride. The methylene chloride eluted was concentrated to 1 ml for gas–liquid chromatographic (GLC) analysis.

DMNA was detected and quantitated with a Varian-Aerograph Model 1740-1 GLC equipped with an alkali flame ionization detector. The GLC column and the conditions for the quantitation and resulting confirmation by mass spectrometry were identical with those described previously (Fiddler et al., 1972b).

RESULTS

The results of the reaction between sodium nitrite and various commercial lecithin samples, or the components of lecithins, are shown in Table I. Considerable quantities of DMNA are formed from DMA and TMA. The components of lecithin—choline, phosphorocholine, and glycerophosphorylcholine—yielded 12 ppm of DMNA, or less. Commercial soy, egg, and vegetable lecithins yielded 0.70–5.40 ppm of DMNA, while a 60% bovine lecithin sample formed 30.76 ppm of DMNA. On purification, however, the DMNA formed from bovine lecithin declined to less than 2 ppm. Only the synthetic lecithin contained a larger quantity of DMNA, 320 ppm.

The lecithin samples were analyzed for free DMA and TMA and the results are also shown in Table I. Substantial quantities of both of these precursors of the nitrosamine are present in all of the samples.

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Table I. Dimethylamine, Trimethylamine, and Dimethylnitrosamine from Lecithin and Its Components

Compound	Weight, mg[a]	Amine contaminants, mg		DMNA[b]	
		DMA	TMA	mg	ppm[c]
Dimethylamine	205.6		N.D.	32.411	157,670
Trimethylamine	269.5	N.D.		2.932	10,876
Choline chloride	636.6	N.D.	N.D.	0.001	0.83
Phosphorocholine chloride	996.8	0.454	2.718	0.013	12.67
Glycerylphosphorylcholine	1172.8	N.D.	N. D.	0.002	1.50
Synthetic lecithin	3343.9	4.414	4.682	1.069	319.70
Bovine lecithin (60%)	5478.0	0.247	33.800	0.169	30.76
Bovine lecithin (purified)	3005.6	N.D.	4.809	0.005	1.66
Egg lecithin	4724.0	0.435	21.305	0.026	5.40
Vegetable lecithin	4399.3	0.431	3.436	0.005	1.02
Soy lecithin (commercial)	4284.5	0.420	6.598	0.003	0.70
Soy lecithin (edible)	4692.5	0.324	1.558	0.010	2.05

[a] 4.56 mmol. [b] Confirmed by mass spectrometry. [c] Milligrams of DMNA/1000 g of compound. N.D., none detected.

DISCUSSION

The formation of dimethylnitrosamine occurs in commercial preparations of lecithin exposed to nitrite, regardless of the source of origin. The nitrite reacts with dimethylamine derived from the choline portion of the molecule. Formation of the nitrosamine, however, may be facilitated by the presence of DMA and TMA arising during the isolation of the lecithin.

The concentration of lecithin used in food products ranges from 0.1 to 3% and may be present in meat at concentrations approaching 0.5%. Under the conditions of our experiments quantitatively determinable concentrations of DMNA could be produced, reaching a level of 3 ppm in a product containing 1% synthetic lecithin. Wrobel and Rendek proposed in a recent patent (1973) the incorporation of vegetable lecithin into pork belly curing solutions, yielding as high as 1% lecithin in the finished bacon. It would be potentially possible to produce approximately 10 ppb of DMNA in such bacon based on the information obtained on vegetable lecithin and presented in Table I.

Thus, the possibilities of adverse reactions from the incorporation of lecithin into products that may be exposed to nitrite at elevated temperatures should be weighed.

Note: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

ACKNOWLEDGMENT

The authors wish to thank Edward Piotrowski for the mass spectral analysis and Central Soya, Chemurgy Division, for the phosphatidylcholine analysis of the lecithins.

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