Reactions of Lipids in Corn with Ammonia

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ABSTRACT

Ammonia has recently been employed in experimental tests for the treatment of corn to inactivate aflatoxin, to control molds, and as a preservative during ambient-air drying of freshly harvested high-moisture corn. When these various ammonia treatments are properly applied, no adverse effects have been found on any of the constituents of whole corn. However, in the work being reported when 0.1% or more ammonia was in contact with corn in the presence of air for more than a few days, irreversible changes occurred in the polyunsaturated lipids that were proportionate to the ammonia concentrations. The extent of the changes were dependent upon: (a) air-to-corn ratio, (b) ammonia concentration, (c) temperature, (d) corn moisture, and (e) time. The changes were characterized by a reduction in unsaturation of the lipid, by incorporation of nitrogen into the lipids, and by a consequent increase in lipid polarity. The change in polarity of these altered lipids rendered them unextractable with the usual fat solvents. Air (oxygen) and an initiating mechanism that occurs naturally in corn were required for the lipid-ammonia interaction to occur. This interaction involved only the polyunsaturated fatty acid moieties and formed a class of nitrogenous derivatives. Corn may be treated with ammonia for purposes of detoxification or preservation for long periods with no detectable adverse effects on the lipid composition, as long as headspace air is kept low during ammoniation. This may be accomplished in any of the following ways: (a) by ammoniating the corn with a very limited headspace, (b) by ammoniating corn in a sealed bin and displacing the air in the headspace with ammonia gas, and (c) by replacing the headspace air with an inexpensive, inert gas such as nitrogen.

INTRODUCTION

Shelled corn has been treated with ammonia to control molds (1,2) especially in low-temperature drying of high-moisture corn (3-6). Ammonia has also been used for detoxification of corn contaminated with aflatoxin (7-9). Ammonia can react with various constituents of the corn depending on temperature, ammonia level, time, and corn moisture. The effects are observed physically by the initial darkening of the pericarp area. Extended exposure of corn to ammonia results in more intense darkening of the germ and a slow darkening of the endosperm (10).

During the routine analysis of ammonia-treated, high-moisture corn from a pilot-scale storage study (5), an unexpected phenomenon was observed. Within 2-3 days after the addition of ammonia to whole corn in the presence of air (oxygen), the fatty acid composition of the corn oil was considerably reduced in both linoleic acid (C18:2) and linolenic acid (C18:3) with eventual loss of three-fourths of the polyunsaturated lipid. The quantity of pentane-hexane extractable lipid also decreased proportionately. In later experimental studies, fatty acid composition of the lipids from ammoniated, aerated corn was found to contain no C18:3 due to rapid reaction with ammonia in the presence of air. The reaction of C18:2 with ammonia was slower and easier to follow, and is the subject of these investigations. The fate of C18:3 will not be considered further in this study.

Laboratory experiments were carried out to study the following parameters of the reaction between C18:2 and ammonia in the presence of air: (a) air-to-corn ratio, (b) ammonia treatment time, (c) corn temperature, (d) corn moisture, and (e) ammonia addition level.

EXPERIMENTAL PROCEDURES

Preparation of Ammoniated Corn Lipid (ACL)

A mixture of shelled yellow dent hybrid corn varieties (Pioneer single crosses), 1974 crop-year, was weighed (100 g) into a 500-ml filter flask equipped with a side tube. Ammonium hydroxide (28%, NH_3 w/w, determined by acidimetry) and water were added to the corn at ambient temperature and a variety of concentrations. The corn was aerated daily by passing air through a ¼-in. OD glass tubing which extended to the bottom of the filter flask. Sufficient air was passed through the flask to replace the headspace gas once daily. Headspace gas analysis indicated that after 4 weeks less aeration was required. Regardless of the combination of parameters, with excess air present during ammoniation of corn, the corn lipid leveled out at about 75% C18:2 alteration. The ACL could easily be extracted from the ground ammoniated corn using the relatively polar Bollman's solvent (benzene-ethanol-petroleum ether, 1:1:1). The total lipid extracted with Bollman's solvent agreed well with the quantity of normal oil extracted from untreated corn using pentane-hexane. The total amount of altered C18:2 in ammonia-treated corn was determined indirectly by methylating the extracted ACL and analyzing it by gas chromatography (GC). The elevated palmitate (C16S) value for each sample was normalized to equal the C16S in the original corn oil which brought about a redistribution of the fatty acid values yielding a value for total altered C18:2 by difference.

Investigation of Effect of Air Concentration on Corn Lipids during Ammoniation

To study the effects of air on corn lipids during ammoniation, two corn samples (25% moisture) were subjected to 1.5% ammonia (corn, dry matter basis) and stored in filter flasks at 38 C as previously described. One sample was aerated periodically (daily at the onset, less frequently after 6 weeks) while the other sample was kept under a nitrogen headspace. The corn was sampled and analyzed after 1, 3, 6, 12, and 26 weeks. The effects of air were also studied by varying the air-to-corn ratio while all other parameters (i.e., % ammonia, temperature, and corn moisture) were equal. Identical corn samples were stored in sealed containers with varying amounts of headspace air with no interim samples taken to disturb the headspace gases. The volumes of headspace air per absolute volume of corn were: 1, 6, 12, 26, 126, and 286.

After 7 weeks, the headspace gas of each container was analyzed by GC. A dual column system was employed; molecular sieves (13X) were used for oxygen and nitrogen analyses and a carbowax 1500 for determining carbon dioxide, ammonia, and water. The columns could be readily changed from series to parallel operation. The color of each corn sample was measured with a Huntercolor Difference Meter, model D-25, and the fatty acid composition of the ACL was determined by GC.

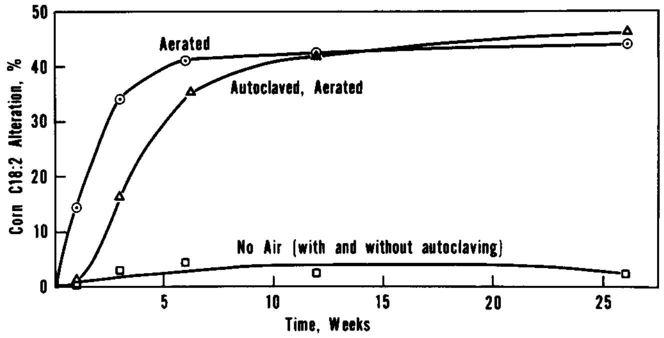

FIG. 1. Effect of air and autoclaving of corn upon degree of C18:2 alteration in ammoniated corn. Ammonia addition level 1.5%, corn moisture 25%, and storage temperature 38 C (corn autoclaved for 30 min at 121 C).

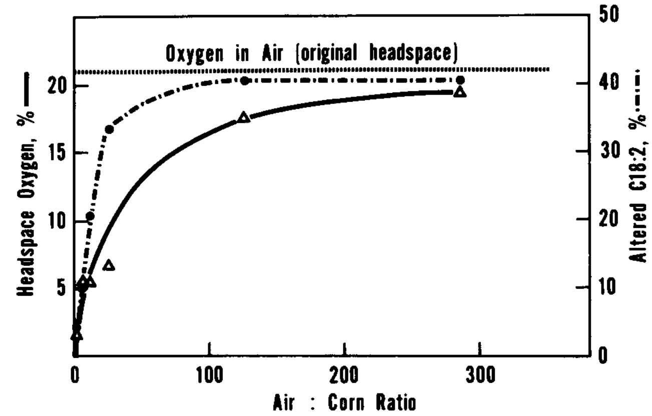

FIG. 2. Effect of air-to-corn ratio upon residual oxygen content of headspace gas and upon altered C18:2 content of ammoniated corn. Ammonia addition level 1.5%, corn moisture 25%, storage temperature 26 C, and storage time 7 weeks.

Investigation of Ammonia Level, Temperature, and Corn Moisture on Corn Lipids during Ammoniation in the Presence of Air

Corn in sealed filter flasks at varying ammonia levels, temperatures, and corn moistures was aerated periodically. Lipid analyses were made by GC after 1, 3, 6, 12, and 26 weeks, except for the corn-moisture experiment which was sampled after 2, 4, 6, 12, and 26 weeks. With all other parameters constant, the following variables were employed: (a) ammonia levels of 0.05, 0.2, 0.5, 1.0, and 2.0%, (b) temperatures of 26, 38, and 52 C, and (c) corn moistures of 11, 25, and 40%.

Analysis of ACL Constituents

At desired sampling intervals, 10 g of ammoniated corn was quickly withdrawn from the filter flask, avoiding significant loss of ammonia. The sample was dried at room temperature for 16 hr (overnight) and ground in a Wiley mill to pass through a 40-mesh screen. The ground corn was further air dried until the odor of ammonia disappeared (4 hr). The sample was slurried with 25 ml of 0.01M hydrochloric acid, filtered, and again air dried. The sample was extracted for 16 hr with Bollman's solvent followed by removal of the solvent on a steam plate under a flow of nitrogen. Methyl esters of ACL were prepared using boron triflouride following the official AOCS method (11).

The fatty acid composition of each sample of ACL was determined on a GC equipped with a 6 ft x ¼ in. column packed with 20% diethylene glycol succinate (DEGS) on Chromosorb W and operated at 200 C.

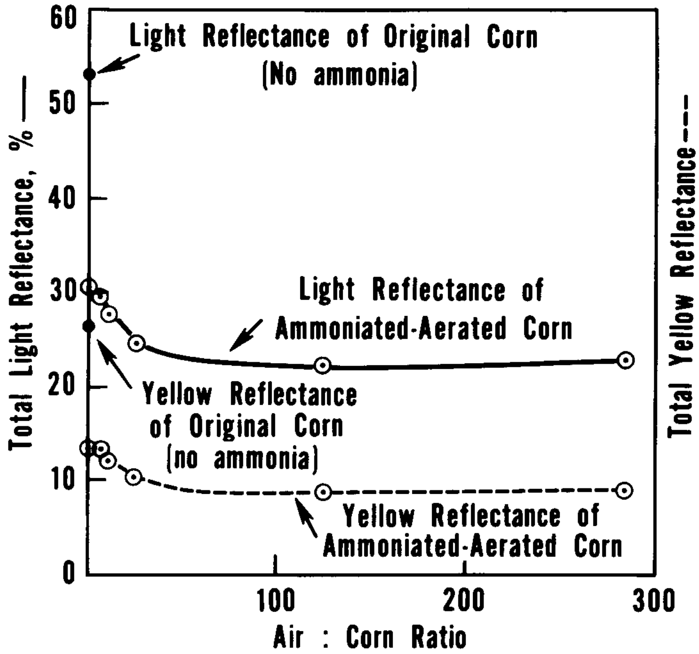

FIG. 3. Effect of air-to-corn ratio upon light reflectance of ammoniated corn.

In an attempt to elucidate the chemical structure of ACL, other GC analyses were conducted with a variety of instruments. Methyl esters of ACL were run on two nonpolar columns (Apiezon L and OV-1) as well as two polar columns (LAC-2-R446 and Silar 5-CP). For gas chromatographic-mass spectrometer (GC-MS) analysis, a Dupont 21-492-1 MS was operated at 70 eV with a source temperature of 220-240 C. Samples for MS were separated by GC using the Silar 5-CP and the OV-1 columns which had very low liquid phase bleed, thus avoiding contamination of the MS.

Thin layer chromatography (TLC) was used for both qualitative identification of lipid materials and, on a large scale, for obtaining samples of unknowns for further structural identification. The TLC plates were Silica Gel G eluted with hexane-ether (50:50) or chloroform-methanol ($CHCl_3$-MeOH) (50:50) solvent mixtures.

Samples were saponified following the official AOCS method (11). Other methods used to prepare methyl esters of ACL were: acid-catalyzed transesterification with 5% hydrochloric acid-methanol and base-catalyzed with 0.1% sodium methoxide-methanol ($NaOCH_3$-MeOH). Recoveries were made by ether extraction of fatty material following the saponification of esterification step, except for one $NaOCH_3$-MeOH catalyzed preparation in which the solvent was removed directly from the esterification flask by a rotary evaporator.

Preliminary separation of this preparation was accomplished using column chromatography by washing the solvent-free material with hexane five times, followed by five washings with benzene. The remainder of the sample was eluted by methanol (MeOH).

RESULTS AND DISCUSSION

Laboratory experiments proved conclusively that ammoniation of corn in the presence of atmospheric oxygen caused a substantial chemical alteration in the C18:2 fatty acid moieties of the corn oil (Fig. 1).

Parameters that Affect Rate of ACL Formation

Independent variables that affect changes in corn lipid include exposure to oxygen, ammonia concentration, tem-

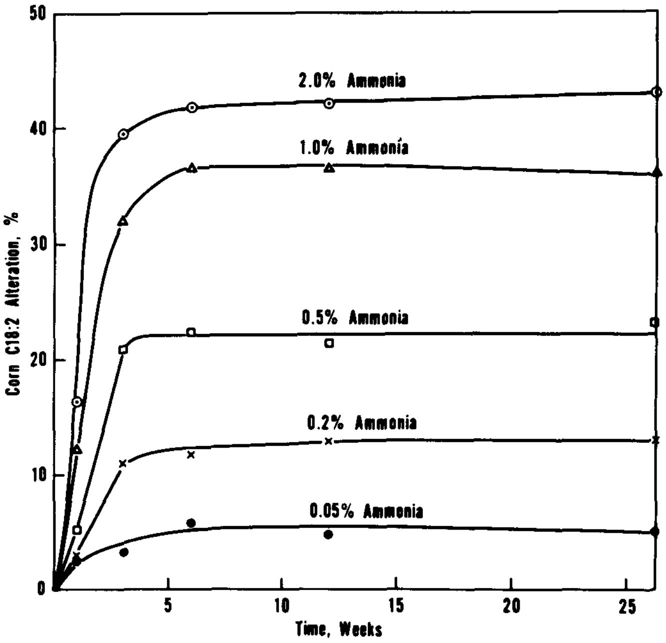

FIG. 4. Effect of ammonia addition level upon degree of C18:2 alteration in ammoniated corn. Corn moisture 25%, storage temperature 38 C.

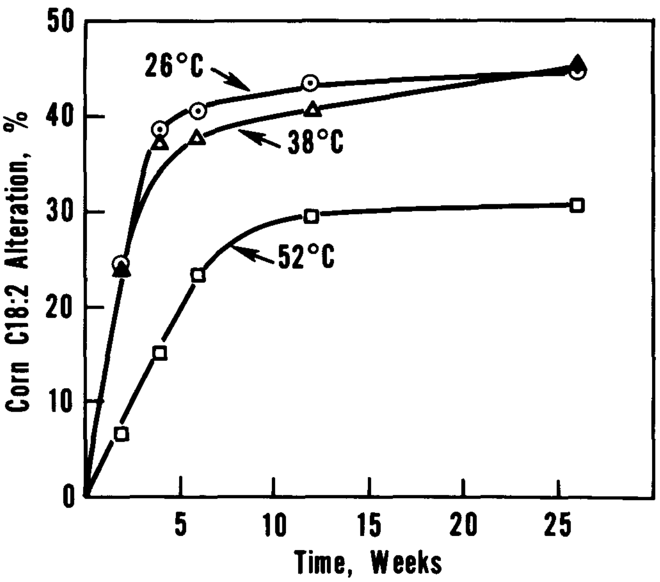

FIG. 5. Effect of temperature upon rate and extent of C18:2 alteration in ammoniated corn. Corn moisture 25%, ammonia addition level 1.5%.

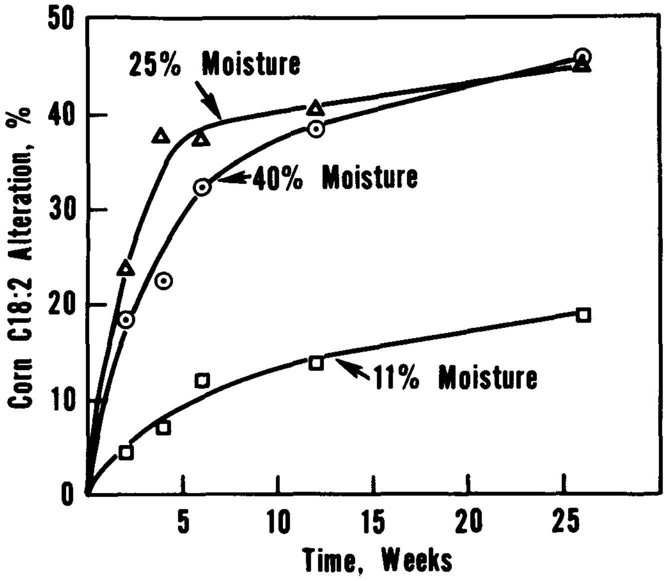

FIG. 6. Effect of corn moisture content upon degree of C18:2 alteration in ammoniated corn. Ammonia addition level 1.5%, storage temperature 38 C.

perature, and moisture content. Figure 2 clearly shows that degree of C18:2 alteration is dependent on quantity of oxygen present in the headspace. The greater the headspace volume, the greater the extent of C18:2 alteration.

This experiment also pointed out other changes that can occur during ammoniation. Corn which was ammoniated with the smaller quantities of air maintained a higher light reflectance (Fig. 3). The total light reflectance (basis:MgO = 100% reflectance) of whole-kernel corn dropped from 54 to 31%, a fractional decrease of 43% during ammoniation in the presence of small amounts of air, whereas the drop was 57% with large amounts of air. The same effect was seen in the absolute (yellow) color of ammoniated corn. The above reflectance data were for samples ammoniated for 7 weeks. The reflectance differential between aerated and controlled atmosphere (nitrogen) samples was much greater for short-time ammoniations (1 week).

The above color reductions are indicative of the changes that occur in Maillard-type browning reactions. During ammoniation of corn in the presence of air, both the browning reaction and the C18:2 alteration are affected by: (a) increasing ammonia concentrations, (b) increased corn moisture, (c) increased temperature, and (d) reaction time. In the absence of air, these factors affect only browning and cause no alteration in the C18:2.

The effect of ammonia concentration was studied at five different levels, but at the same moisture level (25%) (Fig. 4). From 0.05 to 1.0% ammonia, an almost linear relationship exists between the alteration of C18:2 and the concentration of ammonia. In addition, regardless of the ammonia level, the C18:2, oxygen, and ammonia interaction initially proceeded quite rapidly and was essentially complete in 4-6 weeks. Ammonia was strongly detected even after 26 weeks. It would seem that with an ammonia level above 2%, C18:2 alteration should approach the sum total of polyunsaturates in corn oil (60%). However, higher ammonia levels were not used because the maximum amount of ammonia used in the field trial detoxification of aflatoxin-contaminated corn is 1.5% (9).

To study the effects of temperature on ammoniation, three identical samples were stored at three different temperatures (Fig. 5). The interaction of C18:2, oxygen, and ammonia decreased with increasing temperature. This implies the occurrence of competing reactions with other constituents in corn which proceed faster at higher temperatures, leaving less ammonia available for the alteration of C18:2. Again, the initial relatively rapid interaction rate occurs, with near completion in 4-6 weeks.

The effect of moisture content on ammoniated corn lipid was determined by storing corn at three widely varying moisture levels, at 38 C, 1.5% ammonia and sampling at 2, 4, 6, 12, and 26 weeks. The results of these experiments (Fig. 6) indicate that air and ammonia do not have widely divergent effects on corn having moisture levels of 25% or more.' Low-moisture corn samples are least susceptible to C18:2 alteration.

Analytical Characterization of ACL

In addition to GC and light reflectance, several other analyses were made in order to characterize the ACL (Table I). The Wijs iodine value of the ACL was reduced to 70 as opposed to 120 for the control. The conjugated diene content was over fivefold higher (3.7%) than the control. The most unusual result was the relatively high nitrogen content (2.2%) of the ACL, which indicates some type of ammonia addition to the corn triglyceride molecule.

A preliminary investigation of ACL by TLC indicated the presence of (a) mono-, di-, and triglycerides, (b) fatty acids, (c) fatty amides, and (d) a dark-colored material that would not move (some streaking did occur) using conventional fat solvent systems such as hexane-ether. This dark material could be moved with $CHCl_3$-MeOH (50:50) but still streaked badly with few distinct separations.

Identification of amides present in ACL was made on the basis of GC retention characteristics and verified by GC-MS. Two different ACL samples were analyzed and found to contain 1.9% and 1.3% total long-chain amides. The relative proportions of C16S, C18S, and C18U amides in ACL were identical to the fatty acid composition of the ACL.

Column chromatography was used to separate the various components of the Bollman's solvent extracted ACL. Approximately one-third of the ACL was eluted from the column with hexane-ether (50:50) and was composed of unaltered triglycerides and fatty acids. Most of the remaining two-thirds of the corn lipid was eluted from the column with MeOH and was composed almost entirely of altered triglycerides whose fatty acid composition was also reduced in the amount of normal C18:2.

Of primary interest was the dark, MeOH-soluble material which, after transesterification, still contained unaltered fatty acids. The remainder of this transesterified material was the dark, viscous mixture referred to as altered C18:2. With the exception of the amides present, the identities of these compounds are not known.

The interaction between C18:2, oxygen, and ammonia was random with respect to addition of ammonia to the individual C18:2 fatty acid moieties of the corn oil triglycerides. This fact was determined by transesterification and GC analyses. After transesterification, the fatty acid composition of the pentane-hexane extracted triglycerides (i.e., those triglycerides containing only normal fatty acids) was identical to the composition of the remaining normal fatty acids obtained by transesterification of the total triglycerides extracted with Bollman's solvent (i.e., triglycerides containing altered C18:2). This same random effect was observed in the formation of the small amounts of fatty amides described above.

Although a thorough characterization of the chemical structures of ACL have not been compiled, it has been shown that ammonia reacts chemically with double bonds of the fatty acid moieties of polyunsaturated corn triglyceride. The ACL is a mixture of normal triglycerides and a dark, viscous, polar, nitrogen-containing triglyceride fraction which is greatly reduced in unsaturation. The fact that the triglyceride fraction contains nitrogen indicates that the fatty acid moieties which have reacted with the ammonia have not been removed from the glycerol radical. Small quantities of long-chain amides were identified in the ACL. Material balance data indicate that water-soluble components are probably present.

TABLE I

Chemical Analyses on Lipid Extracts from Control and Ammoniated Corn Using Bollman's Solvent

Analysis	Control corn	Ammoniated corn
Unaltered fatty acids	96%	52%
Linoleic acid content	58.5%	20.7%
Iodine value (Wijs)	119.7%	70.3%
Conjugated double bonds	0.7%	3.7%
Saponification equivalent	291	302
Nitrogen (Kjeldahl)	0.005%	2.2%

These findings lend support to our tentative thesis that when corn is treated with ammonia in the presence of air, the ammonia causes excitation of the double bonds in the polyunsaturates which results in conjugation. This reaction could conceivably be followed by the formation of some type of oxygen intermediate such as hydroperoxides. Hydroperoxides decompose to carbonyls that react with ammonia-produced imines. These compounds tend to undergo the Diels-Alder condensation, producing a complex class of nitrogenous adducts formed through inter/intramolecular reaction.

Further studies are being conducted with two primary objectives: (a) to determine more precisely the chemical structures of the nitrogen-containing compounds and adducts, and (b) to identify the naturally occurring factors or enzymes in corn which act as initiating mechanisms for the lipid, oxygen, and ammonia reaction.

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