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Changes in the chemical composition and utilization

of artificially altered corn grain

by

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TABLE OF CONTENTS

INTRODUCTION

Corn grain and corn grain products have become widely accepted as a food source for both ruminant and nonruminant animals. To reduce storage and harvesting losses and to improve rate of gain and efficiency of utilization, many methods of artificially altering corn grain have been proposed. With newer methods of grain harvesting, the amount of grain harvested at moisture contents above the safe storage level has increased; however, these methods have drastically reduced harvesting losses. Many mechanical drying methods suitable for removing the excess moisture have been introduced and widely explored. Drying has reduced storage losses but the nutritive value of the dried grain is variable and depends on moisture content, temperature and time of drying.

As an alternative to drying, ensiling of corn grain at high moisture contents in oxygen-limiting structures, conventional tower silos or horizontal silos provides a satisfactory means of storage. Ensiled high moisture corn, similar to all silage processes, undergoes fermentation. Factors such as moisture content of the grain, temperature and oxygen supply influence the extent of fermentation, the development of molds, and the subsequent nutritive value of the ensiled high moisture corn grain.

Reconstitution, adding water to dry corn grain with subsequent storage under oxygen-limiting conditions, has become another method of improving the nutritive value of corn and other cereal grains. Other methods of artificially altering corn grain include the use of organic acids, moist-heat treatment (steaming followed by flaking) and dry-heat

treatment (microwaving). Moisture content, temperature and time of processing and storage are important variables which influence the nutritive value of the altered corn grain.

Most methods of artificially altering corn grain have been evaluated largely by animal performance. From a practical standpoint, this is the most critical measure of nutritive value; however, differences in animal performance are the net result of changes in the physical and chemical properties of the altered corn grain. Nevertheless, limited information is available on the chemical changes resulting from methods that alter the grain structure.

Therefore, the present studies were initiated to 1) evaluate a modified laboratory silo apparatus to study the effects of fermentation on corn grain, 2) to determine the changes in carbohydrate fractions of artificially altered corn grain at different moisture contents, 3) to determine the changes in nitrogen fractions of artificially altered corn grain at different moisture contents, 4) to determine the changes in in vitro utilization of the carbohydrate and nitrogen fractions of arti- ficially altered corn grain and 5) to study the effects of length of storage on reconstituted corn grain with and without formic acid.

REVIEW OF LITERATURE

A literature review concerning the artificial alteration of cereal grains revealed that numerous reports are available relative to their nutritive value when used in livestock rations. Since several articles (Burroughs et al., 1960; Burkhardt et al., 1969; Schake, Riggs and Butler, 1972) have been published on the feedlot performance of cattle fed artificially altered cereal grains, only those reports directly related to the physical and chemical properties of the grain will be considered.

Many attempts have been made to reduce storage losses of cereal grains harvested at high moisture contents. These losses are especially pronounced in years of late maturity, early frost, early harvest or weather conditions that are unfavorable for natural field drying. The practice of heat drying high moisture grains under forced draft has become a widely used method for eliminating such losses.

Hathaway, Yung and Kiesselbach (1952) reported that weight gains of rats were reduced when corn, dried at temperatures above 60 C, was fed as the source of energy. Nutritive value of the protein was adversely affected by drying temperatures of 72 C and higher. In contrast, Clanton, Hemstrom and Matsushima (1960) reported no differences in digestibility of nutrients in cattle rations containing corn dried at 88 C.

The decrease in nutritive value of artificially dried corn appears to be related to the extent of kernel damage (Emerick, Carlson and Winterfeld, 1961). As demonstrated by Cabell, Davis and Saul (1958), the extent of kernel damage is related to moisture content at harvest, drying temperature, heating time and air flow rate. Their results show that if

sufficient air flow rate is used, corn containing 29 to 30% moisture can be dried at 116 C without any appreciable losses in nutritive value.

Variable results have been reported on the effects of drying on chemical composition. Emerick, Carlson and Winterfeld (1961) reported small differences in the protein, ether extract, crude fiber and ash content of corn dried at 21, 38, 76, 94 and 121 C, none of which appeared to be directly related to drying temperatures. In contrast, works published by Jensen, Terrill and Becker (1960) showed an apparent increase in ether extract and crude fiber values from a drying temperature of 104 C compared with 60 and 82 C. These workers also reported that increased drying temperature did not appear to affect the organic soluble constituents but the starch portion became slightly less susceptible to malt amylase digestion.

Work with the electron microscope has shown that cavities or fissures are formed when corn starch is dried (Whistler et al., 1958). These cavities, which open on the starch granule surface, develop when the starch is dried in air as well as those dried by alcohol dehydration. Since these cavities open on the granule surface, it was assumed that the cavitated granules differ from solid granules in chemical reactivity.

In later work, Whistler, Goatley and Spencer (1959) reported that corn starch dried at 45 C to 33, 21 and 8% moisture contained 25, 35 and 50% cavities, respectively. However, little difference was shown in gelatinization temperatures and viscometric properties. As the moisture content was lowered, these starches showed decreased reactivity to oxidation, sulfation and acetylation, although the extent of cavitation was increased.

In addition to the physical stresses applied to the starch granule during drying, these workers showed that the surface layer of starch molecule seems to become more tightly bonded intermolecularly in the process of air drying. The resulting increase in degree of association gives the starch granule a "case-hardened" shell which influences the rate of diffusion of penetrating chemicals and, consequently, the rate of chemical reactivity. This decrease in reactivity exceeds the compensating effect of increased area due to cavitation and results in reduced total granule reactivity.

Moisture content, storage temperature, oxygen supply and degree of soundness of the grain determine the rate at which most chemical changes occur in stored, dry grain (Zeleny, 1948). Most of the changes are enzymatic in nature and result from the action of the enzymes of the grain itself or of the bacteria or fungi present in the grain. Thus, grain stored at moisture levels and temperatures below which fungi and bacteria proliferate usually exhibits very little enzymatic or respiratory activity and undergoes chemical changes very slowly.

Under certain conditions an increase in the dry weight of grain during storage has sometimes been observed. This increase is due to the uptake of water in the reactions involved in the hydrolysis of starch (Zeleny, 1948). Thus, the dry weight of the products of hydrolysis is greater than that of the original starch. Generally, conditions that favor starch decomposition also favor respiratory activity so that the sugars are consumed and converted into carbon dioxide and water. Under these conditions, which usually occur at moisture levels of 15% or more, the grain loses both starch and sugar and the dry weight decreases. It

is thought that amylase activity may continue after respiration has ceased under conditions where large amounts of reducing sugars accumulate during long periods of storage.

The total protein content of grain is assumed to remain unchanged during storage. However, small, progressive increases in protein content can occur during extended storage (Zeleny and Coleman, 1939). This increase in protein on a percentage basis is no doubt the result of a loss in carbohydrate by respiration.

Proteolytic enzymes in grain and in organisms associated with grain hydrolyze the proteins into polypeptides and finally into amino acids. Until the grain has reached an advanced stage of deterioration, hydrolysis occurs very slowly and cannot be detected by changes that occur in total nitrogen (Zeleny and Coleman, 1939).

Jones, Devine and Gersdorff (1942) reported that during storage the proteins of corn decrease in solubility and _in vitro_ digestibility by pepsin and trypsin. Simultaneously, there occurs an increase in amino nitrogen and a decrease in "true protein." Corn stored at about 12% moisture at 20 C for 2 years showed a decrease of 3.6% in protein digestibility. As well as a decrease in nutritive value, these workers showed that as the length of storage increased, the palatability of ground corn also decreased when fed to rats. This deterioration is assumed to be associated with the changes in protein solubility and digestibility.

As an alternative to heat drying of grains, satisfactory storage of high moisture grains in oxygen-limiting structures, conventional tower silos or horizontal silos has been achieved. Similar to all silage making

processes, ensiled high moisture grains undergo fermentation with acid and ethanol production.

The principles of storing ensiled high moisture grain have been reviewed by Hyde (1965). Moisture, temperature and oxygen are thought to be the most important external variables which control the storage of grain. The development of molds, bacteria and insects during storage can be prevented by reducing the moisture content of the grain, decreasing the temperature and by the exclusion of oxygen. Generally, a moisture content of about 14%, a temperature of 10 C and an oxygen-free atmosphere will inhibit the growth of molds and bacteria. Certain molds, however, will continue to develop at very low oxyten tensions (0.1% oxygen) at higher moisture contents (Bottomley, Christensen and Geddes, 1952).

In addition to the external variables affecting the storage of grains, there are certain so-called internal variables that also affect storage (Hyde, 1965). Grains are generally considered living organisms which respire, thereby producing heat, carbon dioxide and water. The rate of respiration is much more rapid at higher moisture levels, particularly if molds are present. Grain is a hygroscopic material which will absorb or give up moisture until it is in equilibrium with the surrounding atmosphere. This equilibrium is dependent on temperature in that an increase in temperature lowers the moisture content at a given relative humidity. Generally, the moisture content of the grain is regarded as the factor limiting mold growth but it is the relative humidity of the atmosphere that is decisive in that most molds will not grow at relative humidities below 75 percent. Grain, being a granular material, is also a poor conductor of heat. Therefore, except at the periphery, internal

or external fluctuations in temperature result in minor changes in temperature of a grain bulk unless there is induced movement of air to cause the transfer of heat.

In oxygen-free storage, the oxygen originally present is consumed by the microorganisms or by respiration of the grain itself (Hyde and Oxley, 1960). The rate at which oxygen is consumed and carbon dioxide is produced depends on the moisture content of the grain. In general, the higher the moisture content the more rapidly the oxygen supply is exhausted. The carbon dioxide produced initially by aerobic fermentation or respiration has the same volume as the oxygen consumed and is not measured as gases of fermentation (Dexter, 1966). On the other hand, the carbon dioxide produced in excess of the oxygen consumed is the result of alcohol production from anaerobic fermentation and is measured as gaseous loss.

Storage of grains at high relative humidities (high moisture contents) is accompanied by a loss of viability and an increase in acidity (Bottomley, Christensen and Geddes, 1950). The hydrogen-ion concentration tends to increase with length of storage, but because of the buffer action of the proteins and other constituents of the grain, marked changes in hydrogen-ion concentration ordinarily do not occur. Titratable acidity, on the other hand, is likely to increase significantly even in the early stages of storage.

A critical study by Zeleny and Coleman (1938) showed that the acids present in grain consist primarily of free fatty acids produced by the action of lipases on fats, acid phosphates produced by the action of phytase on phytin and amino acids produced by the action of proteolytic

enzymes on protein. In terms of neutralization values, their results show that these acids are present in freshly harvested corn in the approximate ratio of 1:5:8 (free fatty acids:acid phosphates:amino acids). Although all three types of acids increase in quantity when grain is stored at high relative humidities, the free fatty acids increase measurably in the very early stages of storage. As the result of their work, it was concluded that fat acidity alone is a more sensitive index of the effect of storage at high relative humidities than either of the other types of acids present.

Bottomley, Christensen and Geddes (1952) reported that mold count and fat acidity (defined as the number of milligrams potassium hydroxide required to neutralize the free fatty acids from 100 g of grain) of aerated corn increased as the moisture increased while the viability and nonreducing sugars decreased. At any given moisture content, the extent of these changes increased with time of storage. After 12 days, aerated corn stored at 19% moisture exhibited a three-fold increase in mold count, a 35% decrease in viability and little change in fat acidity and nonreducing sugar content. In contrast with corn stored at 32% moisture, mold count increased 100,000 times, viability fell to zero, fat acidity increased approximately four-fold and the nonreducing sugars decreased to one-seventh of their original value. In comparison, nonaerated corn stored for 12 days showed relatively little change in mold count, fat acidity and nonreducing sugar content. However, with nonaerated corn stored at 30% moisture, mold count increased 20 times, viability fell to zero, fat acidity remained essentially unchanged and the nonreducing sugars decreased to approximately one-seventh of their original value. Similar changes in fat acidity

and viability have been reported by Foster, Kaler and Whistler (1955).

Nagel and Semeniuk (1947) reported that the changes in the nitrogen content of mold infected corn reflect the metabolic activities of the fungi present. These workers showed that after 4 weeks storage the total nitrogen content decreased presumably because ammonia was lost, water soluble nitrogen increased 2 to 4 times and insoluble nitrogen decreased indicating that nitrogen was mobilized by the fungi.

To obtain multiple use of storage structures, the process of reconstitution of dry grains, particularly sorghum, has been suggested. Reconstitution involves adding water to air-dry grain followed by storage in airtight structures where fermentation is controlled by the limited air supply. The exact changes which occur during reconstitution are not fully understood, but are thought to be similar to germination (Riggs, 1969).

Germination is characterized by a rapid uptake of water which facilitates the mobilization of reserve material and the utilization of these reserves for axis growth (Ingle, Beevers and Hageman, 1964). In the normal course of germination, the uptake of water by the embryo activates mechanisms for gibberellic acid production. The gibberellic acid then diffuses into the aleurone layer where the production of α-amylase, proteolytic enzymes and enzymes which promote cell wall degradation occurs. Production of these enzymes is necessary for hydrolyzing the starch and protein in the endosperm to an extent used by the growing seedling and is dependent on the presence of the aleurone layer (Van Overbeek, 1966). Thus, in the presence of adequate moisture,

the extent of starch and protein hydrolysis may be decreased if the enzymatic pathways are disrupted by processes which destroy the physical integrity of the whole grain kernel.

The rate of water uptake by dry grain is significantly increased with heated water (Riggs, 1969). In laboratory studies, grain immersed in 66 C water for 1 hr. had the same moisture content as grain immersed in 25 C water for 4.5 hours. However, the actual rate of absorption was significantly different only through the first hour. Tween 20, a surfactant, proved ineffective in increasing water uptake. The initial increase in the rate of absorption influenced the difference in moisture contents for approximately 20 hr., after which there was essentially no difference brought about by temperature.

Data presented by Wagner (1970) indicate that there is a definite advantage in reconstituting sorghum grain to 30% moisture. In vitro evaluations of grain reconstituted to 21, 30 and 33% moisture show dry matter disappearances of 35, 48 and 49%, respectively, as compared to 32% for dry grain. The length of storage time also had a significant effect on in vitro digestibility. Dry matter disappearances of 45, 50, 56 and 58% were reported for dry grain and grain reconstituted to 30% moisture and stored for 5, 10 and 20 days, respectively. Similar optimum conditions for reconstituting sorghum grains have been reported by Hale et al. (1969). Their data indicate that high storage temperatures (38 C) are required to obtain maximum in vitro digestibility.

The physical and chemical changes of the grain which occur during reconstitution are not fully understood. With reconstitution, no gelatinization of starch occurs (Hale, 1971). The increase in water

soluble carbohydrates reported by Ingle, Beevers and Hageman (1964)
indicates that considerable hydrolysis of corn starch occurs during the
uptake of water. However, the increased utilization of sorghum starch
is thought to be the result of disruption of the protein matrix rather
than the disruption of the starch granule (Hale, 1971).

Endosperm protein in corn fills the space between the starch granules
where it appears as a two-component system made up of globular bodies
embedded in an amorphous matrix (Wolf, Khoo and Seckinger, 1969). Solu-
bility of the globular protein bodies in ethanol identifies the granules
as a major site of prolamine deposition (Wolf, Khoo and Seckinger, 1967).
The increase in ethanol soluble nitrogen of reconstituted corn reported
by Sprague and Breniman (1969) indicates that disruption of the protein
matrix occurs during reconstitution. Similarly, increases in soluble
protein found in the endosperm of germinating corn are thought to originate
from the hydrolysis of zein located within the protein matrix (Duvick,
1961).

In the past decade there has been a rapid increase in the alteration
of cereal grains by methods that involve some degree of starch gelatiniza-
tion or damage to the starch granule. These methods include processes
such as steaming, steam flaking and micronizing.

The changes that the starch granule undergoes during gelatinization
have been reviewed by Walker (1966). Starch molecules can be bonded to
each other by hydrogen bonds derived from their own chains or from the
water always present in crystalline starch. In the crystalline regions,
the molecules are bound together so tightly that it is difficult for
foreign molecules to penetrate. In the amorphous regions, however, small

molecules like water can enter the starch matrix and force the chains apart, disrupting the structure. This occurs during the early stages of swelling and at moderate temperatures this phase is reversible and the appearance of the starch granules is unchanged if they are cooled and dried. If the swelling process is allowed to continue with the aid of heat and moisture, an irreversible change suddenly takes place in the starch granule. At this stage, granule size increases many times, some of the starch is solubilized, most of the crystallinity of the granule is destroyed and the starch is said to have become gelatinized. After gelatinization, starch granules continue to take up water in a third swelling stage and eventually become pastes which have even more diffuse starch chain networks. If the pastes are cooled, they form gels.

The loss of birefringence is the most convenient method of measuring the end point for gelatinization of purified starch (Seib, 1971). However, measurement of gelatinization of starch in cooked grain is more difficult than for purified starch since the end point is not easily defined. In addition to loss of birefringence, several other methods have been used to measure the extent of gelatinization. Johnson, Matsushima and Knox (1968) reported close relationships between loss of birefringence, increased water uptake and decreased specific gravity for steam-flaked corn. Changes in X-ray diffraction patterns, sorption capacity, solubility and viscosity behavior have also been used to determine the extent of gelatinization (Sair, 1967).

Sullivan and Johnson (1964) reported that gelatinization of starches can be readily measured by beta-amylolysis in complex natural systems where measurement of loss of birefringence is difficult and uncertain.

Their work suggests that loss of birefringence is dependent on changes from the initial state of the raw starch granules which is generally unknown, whereas measurement of the degree of gelatinization by beta-amylolysis is dependent only on the state of the starch at the time of sampling. Pfost (1971), however, compared beta-amylolysis with a microscopic method which determines the percentage of starch granules stained by Congo red dye, and reported that the microscopic method indicates complete starch gelatinization before the enzyme method indicates complete starch breakdown.

The degree of gelatinization, as measured by increased susceptibility to β-amylase, is dependent on processing pressure and the degree of flaking (Osman et al., 1966). Steaming at atmospheric pressure or at a pressure of 1.4 kg/cm^2 for 1 min. without flaking decreased in vitro starch digestion of both milo and barley. Under these conditions, digestibility increased with degree of flaking or flake flatness. Steaming at pressures of 4.2 or 5.6 kg/cm^2, however, increased starch digestion over the untreated grain. At these pressures, flaking significantly (P<.05) increased starch digestion. Further increases in starch digestion occurred with increased degree of flaking.

Frederick, Theuer and Hale (1968) reported that in vitro starch digestion of steamed milo is affected by the pressure plate temperature and pressure applied during flaking. The critical plate pressure for improving starch digestion was approximately 140 kg/cm^2 at room temperature. Increased plate pressure to 1400 kg/cm^2 did not improve digestion. When the pressure plates were heated to 98 C, the critical pressure decreased to approximately 70 kg/cm^2.

In vitro starch digestion is also affected by the interaction of moisture content, pressure and steam processing time (Liang et al., 1970). Susceptibility of corn and sorghum starch to enzymatic attack was increased when both grains were pressure cooked at 6.0 kg/cm^2 compared to 5.3 and 3.5 kg/cm^2 for 1 minute. Addition of 10% moisture resulted in an increased response at both the 1 and 10 min. processing times. When 20% moisture was added, an increase in starch digestion was observed at 1 min. compared to a decreased response at 10 minutes. At the higher moisture level, increased processing time appeared detrimental to the corn starch.

As measured by loss of birefringence and changed viscosity, starch granules of microwaved sorghum grain are completely gelatinized and extensively swelled but are not as susceptible to enzymatic attack as steam-flaked grain (McNeill, Potter and Riggs, 1970). Their data indicate that the degree of gelatinization is not the only factor influencing susceptibility of starch to enzymatic attack. The high temperatures and dry heat used in the microwaving process have a possible detrimental effect on starch availability.

Relative to the effects of gelatinization on the starch granule, little emphasis has been placed on the effects of gelatinization on the protein constituents of cereal grains. McNeill, Potter and Riggs (1970) suggest that the intense dry heat used in microwaving could denature the poorly soluble proteins in the endosperm to make them even less soluble. On the other hand, steam flaking is thought to have a beneficial effect on protein solubility. Histological studies suggest that hydration of the protein matrix results in a disruption of the protein integrity and thus increases solubility (Hale, 1971).

Early observations indicated that increased digestibility was the
primary factor causing increased utilization from artificially altered
cereal grains. Buchanan-Smith, Totusek and Tillman (1968) reported
higher digestion coefficients for dry matter, organic matter, nonprotein
organic matter and energy for reconstituted and steam processed grain
sorghum compared to dry grain when fed to cattle. These increases were
of the magnitude of 7 percent. Digestibility of starch was not affected
by treatment, but when starch and reducing sugars were combined,
digestibility of the combined fractions was higher for the reconstituted
and steam processed grains.

Similar improvements in digestibility of artificially altered sorghum
grain, particularly with cattle, have been reported by Husted et al. (1968)
and Riggs et al. (1970). In contrast, Parrott et al. (1969) with barley
and Cornett, Sherrod and Albin (1971) with wheat found little or no
improvement in digestibility of artificially altered grains compared with
dry rolled grains.

Trei, Hale and Theurer (1970) studied the effect of artificially
altering cereal grains on in vitro gas production. High correlations were
found between gas production and in vitro dry matter disappearance, total
volttile fatty acid production and in vitro starch digestion. Observations
on the relationship of cooking pressure, degree of flaking and gas produc-
tion agreed closely with the enzymatic starch digestion data reported by
Osman et al. (1966).

In vitro starch utilization and rumen digestion of dry-ground,
reconstituted, steam-flaked and microwaved sorghum grain were investigated
by McNeill, Potter and Riggs (1970). Microbial gas production, total

carbohydrate digestibility and insoluble dry matter digestion were greatest for the steam-flaked grain followed by reconstituted and dry-ground with microwaved grain being the lowest. Starch digestion, whether total, ruminal or postruminal, was significantly (P<.05) greater for the reconstituted and steam-flaked grains compared to the dry-ground and microwaved grains. The major portion of the digested starch was apparently hydrolyzed in the rumen of steers fed the reconstituted and steam-flaked grain. Postruminal starch digestion in steers fed dry-ground and microwaved grain failed to overcome the reduced ruminal digestion.

Although differences in the total and ethanol soluble carbohydrates, starch content and rumen solubility occurred with the different processing methods, the magnitude of the differences was not great enough to account for the large differences observed in the ruminal and postruminal carbohydrate utilization.

Potter, McNeill and Riggs (1971) compared the utilization of protein from dry-ground, reconstituted, steam-flaked and microwaved sorghum grain. Although differences in the quantity of nitrogen reaching the abomasum of steers fed the processed grain were not significant (P>.05), abomasal protein from steers fed microwaved grain contained significantly (P<.05) more feed protein and less bacterial protein than that from steers fed reconstituted or steam-flaked grain, indicating differences in the extent of ruminal protein degradation. Differences in true nitrogen digestion were not significantly (P>.05) different among treatments; however, abomasal protein from steers fed reconstituted grain was slightly more digestible. Enhanced ruminal breakdown of the proteins from reconstituted and steam-flaked grain compared to dry-ground and microwaved grain are

consistent with observations of ruminal starch digestion (McNeill, Potter and Riggs, 1970).

These data (McNeill, Potter and Riggs, 1970; Potter, McNeill and Riggs, 1971) indicate that reconstitution and steam flaking of sorghum grain enhance both starch and protein hydrolysis in the rumen while microwaving apparently reduces ruminal action on both starch and protein. With the high temperatures and dry heat used in the microwaving process, it is possible to produce starch derived dextrins which are resistant to enzyme action but are fairly water soluble (Walker, 1966). Also, the intense dry heat could have denatured the poorly soluble endosperm proteins to make them even less soluble. Thus, the effect of starch gelatinization with microwaving, as measured by increased enzyme suscepti-bility, could have been offset by the deleterious effect on the insoluble protein matrix which encapsulates the starch granules.

The starch from reconstituted grain shows no gelatinization (Hale, 1971); however, its utilization is higher than for dry-ground or microwaved grain. This indicates that it may not be necessary to destroy starch integrity but to disrupt the substructure of the kernel to release the starch granules from within the protein matrix, permitting easier access to the starch granules by bacterial or animal enzymes. It may be that reconstitution and steam flaking result in a more soluble protein, permitting easy attack of the starch by rumen microorganisms. It is also possible that the improved starch utilization resulting from steam-flaked grains is independent of the effects on the protein matrix surrounding the starch granule.

SECTION I:

A MODIFIED LABORATORY SILO UNIT FOR

STUDYING THE FERMENTATION OF CORN GRAIN

Introduction

Many types of laboratory silos have been used for silage research, including the evacuated polyethylene bag (Danley and Vetter, 1971), the glass bottle fitted with a mercury valve (MacPherson and Violante, 1966) and the test tube silo fitted with a mercury valve (McDonald, Henderson and MacGregor, 1968). Under optimum conditions these laboratory silos will produce silage of excellent quality and permit the investigation of chemical and bacteriological changes during the fermentation process. Their use, however, is limited in that volatile and seepage losses that accompany fermentation cannot be determined.

The fermentation apparatus used by Barnett (1952) provides for the measurement of volatile losses but the material being fermented is not in the environment that is assumed to exist in larger silos and seepage losses cannot be determined.

Researchers at the University of Minnesota (Otis, Pomroy and Gawreluk, 1959) developed a laboratory silo unit to control external conditions and other variables that affect fermentation and to provide for the measurement of volatile and seepage losses. Volatile gas collection and quantitation by displacement in acidified water is hindered by gas absorption and dissolved gas exchange. A more direct method of measuring volatile losses would also eliminate the need for the complex syphon system of the Minnesota laboratory silo unit.

In the present study, the temperature and pressure regulating systems, the component parts of the silos and the gas collection system of the Minnesota laboratory silo unit were modified to eliminate some of its

complexities and to provide for a more direct measurement of the volatile losses of fermentation.

Materials and Methods[1]

The design of the laboratory silo unit, described herein, is such that the effects of light, temperature and pressure on the fermentation process can be studied either simultaneously or independently. The effects of other variables, such as moisture content or preservatives, can also be determined.

The exterior of the thermostatically controlled cabinet housing nine miniature silos is shown in figure 1.1. To the left of the air circulating duct is the master control fuse box and the switches controlling the heaters and interior lights. Pilot lights indicate which heaters and lights are in operation. Blackout curtains exclude light. Removable plexiglass panels enclose the cabinet.

The interior of the cabinet is shown in figure 1.2. The silos consist of gas-tight plexiglass cylinders, each having a capacity of approximately 2 kg of wet material. The pressure piston pushed upward by the piston rod from the metal air cylinder below can be seen through the plexiglass. Juice collection bottles, connected by tygon tubing to the pressure piston above, are located between the metal air cylinders.

A schematic drawing of one silo unit with its auxiliary equipment is shown in figure 1.3. The silo is centered on the top vent plate which is

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[1]The laboratory silo unit was constructed by the Iowa State University Instrument shop, under the supervision of A. J. Wunderlich.

permanently attached to the upper frame channel. Pressure acting on the enclosed sample holds the silo against the top vent plate. Air displaced by the pressure piston and gas generated during fermentation first passes through the gas collection plate into the top vent plate and then down the gas manifold. The pressure piston is designed so that juice and gas generated from the bottom of the sample are directed to the juice collection bottle, where the juice accumulates and the gas passes into the lower portion of the gas manifold. This gas, along with the gas from the top of the silo, passes into the two gas absorption bottles. The pressure piston is easily removed from the piston rod which facilitates attaching and removing the silo unit.

Compressed air from the air manifold enters the air cylinder through the inlet port and forces the piston rod upward applying pressure on the sample. Air above the air pressure piston within the air cylinder escapes through the exit port on the top of the air cylinder. Air is released to the atmosphere by the pressure release valve when the silo is removed.

The component parts of each silo are shown in figure 1.4. Two ports are provided in the top vent plate. One port is used for circulating a gas of known composition through the space above the sample. The gas manifold is attached to the other port. The gas collection plate facilitates the gas collection and exposes the top of the sample to the circulating gas. The juice collection plate fits on top of the pressure piston and facilitates collection of juice and gas at the bottom of the sample. The 1 mm rubber gasket between the upper part of the silo and top vent plate and the 6 mm O-ring surrounding the pressure plate provide an airtight seal.

The temperature within the cabinet is regulated by controlling the ambient air temperature. Seven individually controlled heaters (two 500 watt strip heaters, three 100 watt and two 150 watt screw-in heaters) are thermostatically regulated on a 0.5 C temperature differential. The air circulating fan operates continuously and the heaters cannot operate unless the fan is in operation. No cooling damper is provided.

The circulating gas and pressure regulating end of the cabinet is shown in figure 1.5. The syphon system of the Minnesota laboratory silo apparatus (Otis, Pomroy and Gawreluk, 1959) was replaced by the present circulating gas system. A gas of known composition passes through two filters to remove water vapor and carbon dioxide into the circulating gas chamber. The circulating gas is supplied to the nine miniature silos either simultaneously or independently by regulating the release valves on the circulating gas chamber. The circulating gas enters the top portion of the silo and the juice collection bottle and passes on into the gas manifold and the gas absorption bottles eliminating the need for maintaining a vacuum in the upper part of the gas collection bottles.

Compressed air (5.62 kg/cm^2) passes through a filter, which removes any water vapor, to the pressure regulators where the pressure is reduced. Each pressure regulator controls the air supply to three air cylinders. Pressure can be maintained with a 4.32 g/cm^2 differential.

To evaluate the modified laboratory silo unit, two experiments were conducted. In both experiments, reconstituted corn grain was fermented under conditions that would result in varying rates of volatile gas production and mold growth. If mold growth occurred in the untreated reconstituted corn, under the anaerobic conditions of the second

experiment, the silos would not be airtight since mold growth should be inhibited with the fermentation additives.

Experiment I

Nine 3 kg samples of dried shelled corn (12% moisture) were randomly assigned to three groups of three samples each. The three groups were reconstituted with distilled water at 4 C to either 20, 25 or 30% moisture. The nine samples of reconstituted shelled corn were assigned at random to one of the nine silos in the laboratory silo unit and ensiled for 35 days at 32 C under a pressure of 527 g/cm^2. Compressed air was passed through each silo for 2 min. twice daily to flush the gaseous products of fermentation into the gas absorbing bottles and to create an aerobic environment. A similar gas absorbing unit was used as a control. The evolved carbon dioxide and volatile nitrogen losses from fermentation were absorbed in 1 N KOH and 4% H_3BO_3, respectively.

Fresh and ensiled high moisture corn grain samples were freeze-ground as described by Danley and Vetter (1971). Duplicate samples were analyzed in the freeze-ground state for dry matter by toluene distillation (DeWar and McDonald, 1961) and total nitrogen by the standard Kjeldahl method (A.O.A.C., 1965). The absorbed carbon dioxide was determined by adding 30 ml 2 N $BaCl_2$ to 20 ml of the potassium hydroxide solution to precipitate the absorbed carbonate. The excess potassium hydroxide was neutralized with 0.5 N HCl to the phenolphthalein end point. The milliequivalent weight of carbon dioxide was calculated as the difference between the milliequivalent weights of potassium hydroxide and hydrochloric acid (A.O.A.C., 1965). Absorbed volatile nitrogen was determined on an aliquot

of the boric acid solution by titration with 0.1 N HCl to the methyl red-methylene blue end point (Pierce and Haenisch, 1955).

The data were analyzed statistically by least squares analysis of variance for a completely randomized design as outlined by Snedecor and Cochran (1967).

Experiment II

Nine 3 kg samples of dried shelled corn (12% moisture) were reconstituted to 35% moisture with distilled water at 4 C and randomly assigned to three treatments of three samples each. The three treatment groups were: 1) reconstituted shelled corn with no additives, 2) reconstituted shelled corn plus a commercial fermentation inhibitor and 3) reconstituted shelled corn plus a commercial mold inhibitor containing 20% propionic acid. Both additives were added as dry powders to the reconstituted shelled corn at the rate of a g of additive per kg of corn on a fresh weight basis. Argon was used as the circulating gas. The conditions of fermentation and analysis of the initial and ensiled samples and gaseous products were the same as those described for Experiment I.

Results and Discussion

The results of Experiments I and II are shown in table 1.1. No significant (P<.05) differences in dry matter (DM) or total nitrogen (TN) losses occurred among the three silos within each treatment for both Experiments I and II. Values for DM losses are similar to those previously reported by Dexter (1966). In both experiments, differences in carbon dioxide and volatile nitrogen losses among the silos within treatments

approached significance (P<.05). A large part of the variation in carbon dioxide and volatile nitrogen losses appeared to be due to factors inherent in the fermentation process since differences among silos could not be attributed to the methods of analysis and no gas leaks in the gas collection system could be detected. A close relationship between TN and volatile nitrogen within silos was observed and indicated that small quantities of nitrogen were lost from the gas collection system.

At 32 C, the temperature within the modified laboratory silo apparatus did not vary more than 0.5 C at any location in the thermostatically controlled cabinet during each 35-day fermentation period. The same degree of temperature control also existed at other temperature settings used in preliminary tests. Air pressure supplied to the air cylinders was maintained with a 4.32 g/cm^2 differential regardless of pressure setting. There appeared to be no difference in the degree of air pressure regulation among the three pressure regulators which control the three groups of air cylinders.

In Experiment I, the growth of mold was present on the upper and lower surfaces of all silos after 7 days. At the end of 35 days fermentation, the mold had spread throughout the three silos containing shelled corn reconstituted to 30% moisture which corresponds with the highest DM and TN losses. Similar increases in DM and TN losses with increasing moisture content have been reported for corn grain (Bottomley, Christensen and Geddes, 1950). No visible evidence of mold growth was present in Experiment II at any stage during the 35-day fermentation period which indicates that anaerobic conditions were maintained in the silos containing the untreated reconstituted shelled corn.

In both experiments, DM, TN, carbon dioxide and volatile nitrogen losses were directly related and are the result of the metabolic activities of the microorganisms present in the ensiled corn grain. No explanation can be inferred from the data for the higher DM and TN losses with the fermentation and mold inhibitor additives.

The data obtained in this study indicate that the modified laboratory silo unit can be used to study the fermentation of corn grain. Variations in the temperature and pressure regulation are similar to those of the Minnesota laboratory silo unit and indicate that these variables can be maintained within narrow limits. The modifications of the component parts of each silo appeared not to affect the maintenance of an anaerobic environment within each silo. Although the absorption and quantitation of the volatile losses of fermentation were variable and differences among the three silos within treatments approached significance (P<.05), some improvement over the Minnesota laboratory silo unit occurred (Otis, Pomroy and Gawreluk, 1959). Absorption of the volatile losses of fermentation and the circulating gas system offer the advantage of exposing the upper and lower surfaces of the ensiling material to a gas of known composition without correcting for the volume of the circulating gas.

The modified laboratory silo unit can also be used for the fermentation of other cereal grains and forages. The design of the unit is such that the effects of moisture content, temperature, pressure, light, additives and time on the fermentation process can be studied. The modified laboratory silo unit is also adaptable for use with temperature and pressure recording devices and other monitoring units.

Summary

Two experiments were conducted to evaluate a modified laboratory silo apparatus. The design of the laboratory system is such that the effects of light, temperature and pressure on fermentation can be studied either simultaneously or independently.

The heating system of the laboratory silos described herein was modified in that no cooling mechanism was included to control temperature. Temperature within the thermostatically controlled cabinet varied 0.5 C regardless of temperature setting.

Air pressure was maintained with a 4.32 g/cm^2 differential with the present pressure control system. There appeared to be no difference in the control of air pressure among the three groups of air cylinders.

The evolved carbon dioxide and nitrogen gases from fermentation were flushed into absorption bottles by a circulating gas system. This eliminated the complex syphon system used in the Minnesota silo unit. Differences in carbon dioxide and volatile nitrogen losses between silos within treatment approached significance ($P<.05$). There were no significant differences ($P>.05$) in dry matter and total nitrogen loss among the three silos within each treatment.

In both experiments carbon dioxide evolution was directly related to dry matter and nitrogen loss. A direct relationship also existed between dry matter and nitrogen loss. Larger losses of dry matter and nitrogen occurred when reconstituted shelled corn was ensiled under aerobic conditions.

Figure 1.1.   Laboratory silo unit containing nine miniature silos
              enclosed in a thermostatically controlled cabinet.
              This is the air temperature control end of the unit.
              Blackout curtains exclude light

AIR
CIRCULATING
DUCT

HEATERS
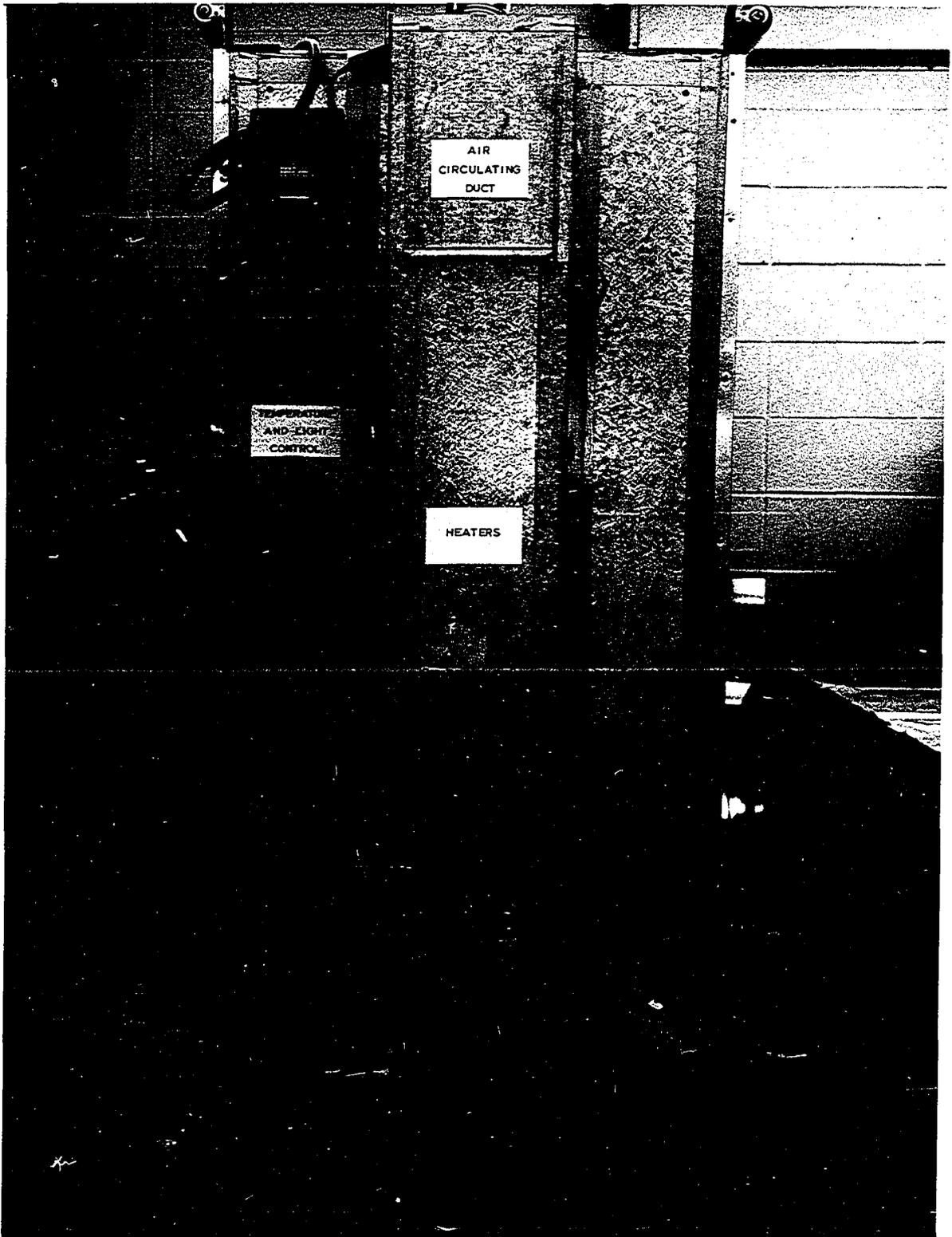
Figure 1.2. Interior of the laboratory silo unit containing plexiglass silos with high moisture corn samples under pressure of pistons actuated by air cylinders below. Juice collection bottles are below each silo. Air pressure regulators are also shown
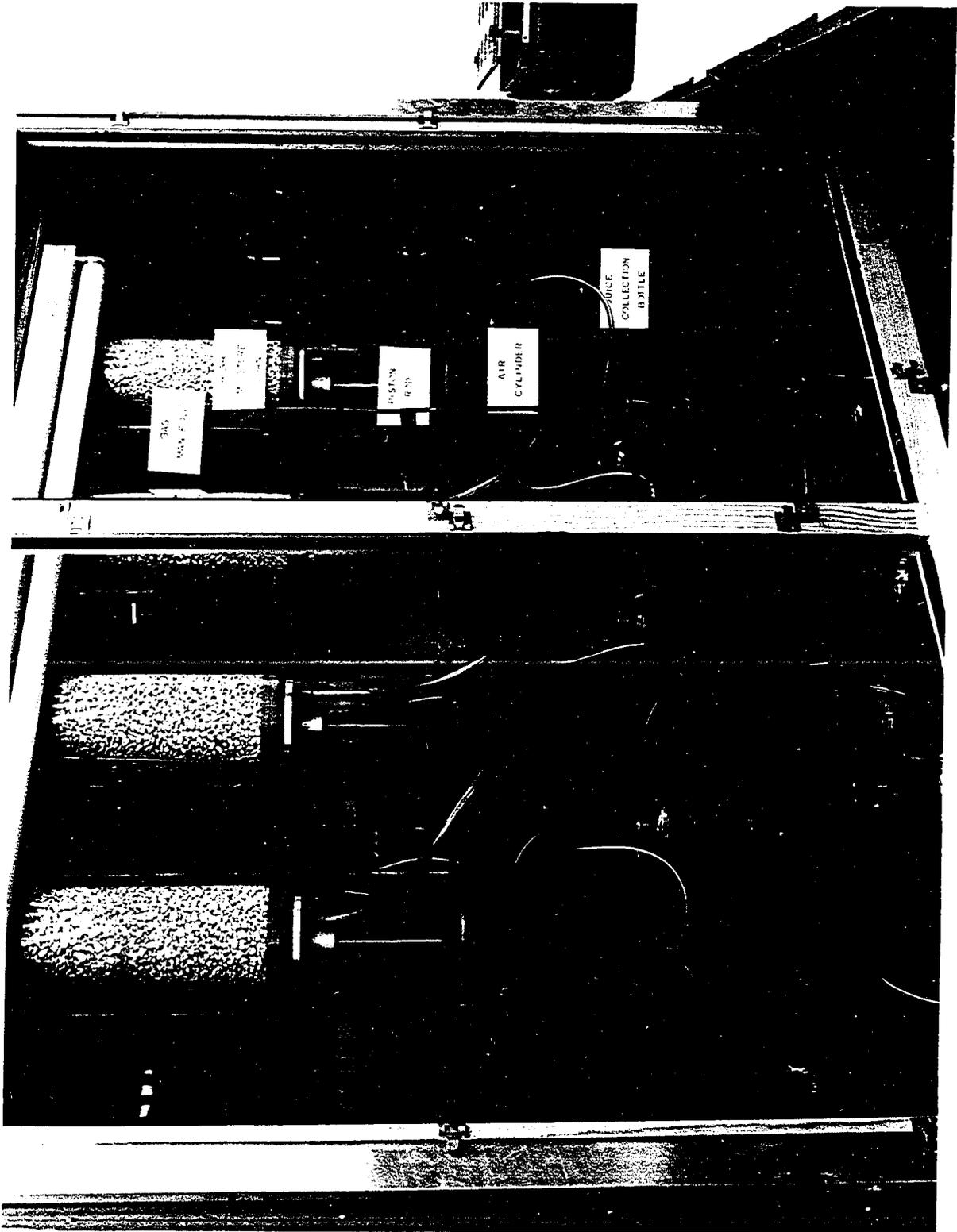
Figure 1.3.   Schematic drawing of one silo unit with its
             auxiliary equipment

CIRCULATING GAS MANIFOLD

VENT AND GAS COLLECTION PLATE

SAMPLE

PLEXIGLASS SILO

JUICE AND GAS COLLECTION PLATE

PRESSURE PISTON

PISTON ROD

AIR PRESSURE PISTON

STEEL AIR CYLINDER

CONNECTION TO AIR MANIFOLD

AIR INLET VALVE

BUNSEN VALVE

UPPER FRAME CHANNEL

GAS MANIFOLD

TUBE COUPLING

JUICE AND GAS COLLECTION TUBE

ALIGNMENT BAR

JUICE COLLECTION BOTTLE

LOWER FRAME CHANNEL

PRESSURE RELEASE VALVE

GAS INTAKE TUBE

GAS COLLECTION BOTTLES

BOTTLE SHELF

Figure 1.4. Component parts of each laboratory silo. The top vent plate
            contains parts for the circulating gas and gas manifold.
            The gas collection plate is placed above the sample. The
            juice collection plate rests on the pressure piston and
            contacts the bottom of the sample. The 1 mm rubber gasket
            between the upper part of the silo and the 6 mm O-ring
            surrounding the pressure plate provide an airtight seal
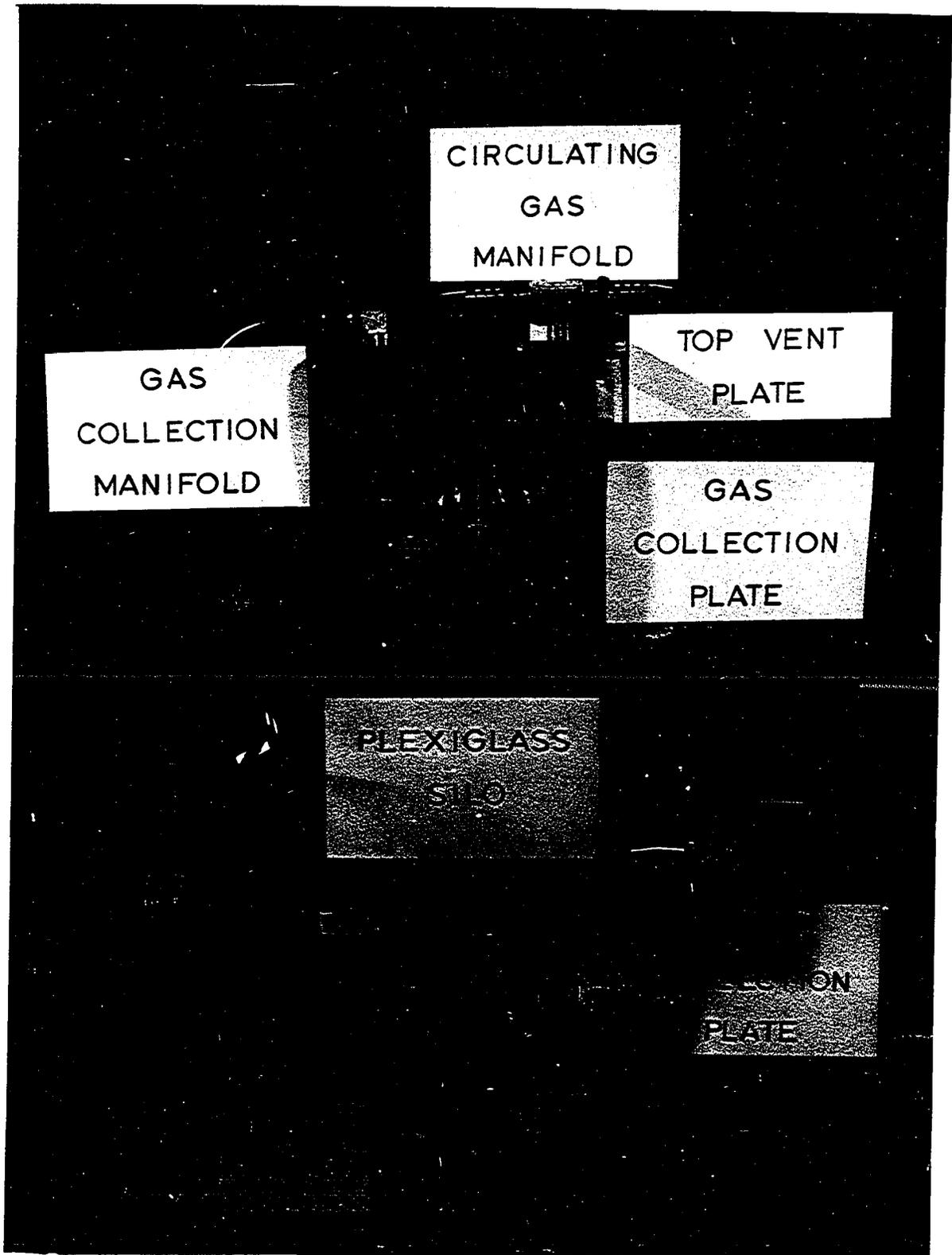
CIRCULATING GAS MANIFOLD

GAS COLLECTION MANIFOLD

TOP VENT PLATE

GAS COLLECTION PLATE

PLEXIGLASS SILO

...CTION PLATE

Figure 1.5. Circulating gas and pressure regulating end of the
            laboratory silo unit. Air pressure controls for the
            three units are located on the exterior of the thermo-
            statically controlled cabinet. The circulating gas is
            passed through two filters before it enters the
            circulating gas chamber. Release valves on the
            circulating gas chamber control gas flow to the nine
            silos

CIRCULATING GAS CHAMBER

TEMPERATURE THERMOSTAT

AIR PRESSURE CONTROL

CIRCULATING GAS FILTER

DRIERITE

AIR FILTER

ASCARITE
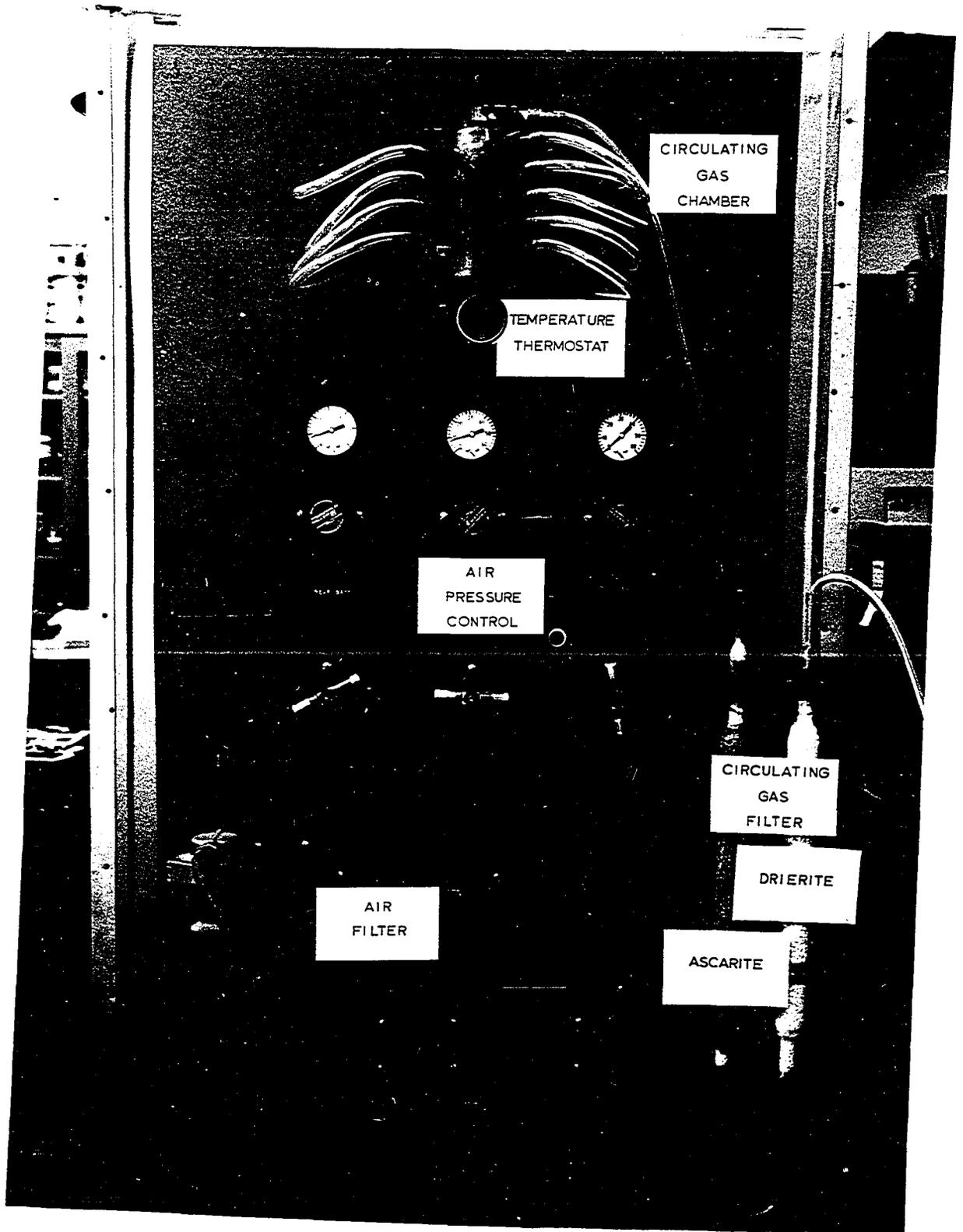
TABLE 1.1. DRY MATTER (DM), NITROGEN (N) AND GASEOUS LOSSES
OF FERMENTED RECONSTITUTED SHELLED CORN[a]

Treatment	Experiment I			Experiment II[b]		
	20%	25%	30%	C	FI	MI
Initial weight, g	2670	2551	2526	2992	3028	3018
Final weight, g	2623	2507	2487	2955	2971	2938
Loss, %	1.76	1.72	1.54	1.23	1.88	2.65
Initial DM, %[c]	80.5	75.6	70.0	64.1	64.0	64.3
Final DM, %[c]	78.5	73.3	68.0	63.5	62.3	62.5
DM loss, %[c,d]	4.21	4.67	5.15	2.26	4.60	5.35
Initial N, mg/g[c]	13.0	13.2	13.5	15.6	15.4	15.4
Final N, mg/g[c]	13.4	13.4	13.7	15.7	15.6	15.4
N loss, %[c,d]	1.35	2.50	3.82	1.38	3.37	4.86
CO_2 loss, mg/g[c,e]	8.68	9.05	10.82	4.21	9.85	11.05
Volatile N, μg/g[c,e]	175	308	514	207	512	732

[a]Values expressed as averages of the three silos per treatment.

[b]Control, fermentation inhibitor and mold inhibitor, respectively.

[c]Values expressed on dry matter basis.

[d]No significant differences (P<.05) among silos within treatments.

[e]Values between silos within treatments approached significance (P>.05).

SECTION II:

DRY MATTER AND NITROGEN LOSSES AND CHANGES IN THE

CARBOHYDRATE FRACTIONS OF ARTIFICIALLY ALTERED

CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS

Introduction

Improvement in the utilization of artificially altered cereal grains is generally attributed to changes in the carbohydrate portion of the grain. Data reported by Whistler, Goatley and Spencer (1959) indicate that a marked decrease in reactivity of corn starch granules to oxidation and absorption occurs as the moisture content is reduced from 60 to 8% moisture by drying. Although the principles of storing ensiled grains have been reviewed by Hyde (1965), little emphasis has been placed on the effect of fermentation on the carbohydrate constituents of cereal grains. The changes in the starch granule that occur during reconstitution and subsequent storage in an airtight structure are thought to resemble those occurring during germination (Riggs, 1969) and result in no gelatinization of the starch granule (Hale, 1971). Formic acid additions to grasses have resulted in lower dry matter and nitrogen losses due to ensiling than untreated silages (Barnett, 1954; Waldo et al., 1969). Data are limited on the fermentation of cereal grains with formic acid additions.

Increased susceptibility of cereal grain starch to beta-amylolysis has been reported for microwaved sorghum (McNeill, Potter and Riggs, 1970) which indicates that gelatinization of the starch granule occurs. The effect of added moisture on the susceptibility of corn and sorghum starch to enzymatic attack has been reported by Liang et al. (1970); however, there are no known reports on the effect of different levels of endogenous moisture on the susceptibility of microwaved and steamed starches to enzymatic attack.

The objectives of this study were to determine the effects of method

of alteration and initial moisture level on dry matter and nitrogen losses
and changes in the carbohydrate fractions of corn grain.

Materials and Methods

Field corn (Pioneer 3570) planted at the rate of 50,000 plants/ha
was harvested at 22, 18 and 16% moisture with a picker-sheller from a
common experimental plot. Prior to planting, 280 kg/ha of nitrogen was
applied. At each harvest, nine 3 kg samples of shelled corn were taken
and assigned at random to three fermentation treatments (untreated-ensiled,
dried reconstituted-ensiled and formic acid reconstituted-ensiled). Corn
harvested at 16% moisture and assigned to the untreated-ensiled treatment
was reconstituted with distilled water at 4 C to 26% moisture to facilitate
fermentation. Samples to be dried and reconstituted were dried to 12%
moisture in a forced-draft oven at 115 C, then reconstituted to 26%
moisture with distilled water at 4 C. Formic acid (88% solution) was
added at 0.5% of the fresh weight during reconstitution of the corn grain
to 26% moisture. Because of variation in the rate of water uptake by
intact corn grain of different moisture contents, over and under recon-
stitution occurred.

Fermentation was studied using the laboratory silo apparatus
described by Otis, Pomroy and Gawreluk (1959) as modified by Danley and
Vetter (1972a). The three fermentation treatments were assigned at random
to the nine, airtight plexiglass silos each having a capacity of about
3 kg high moisture shelled corn. A constant pressure of 527 g/cm^2 was
maintained on the corn mass. Filtered argon was passed through each silo
for 2 min. twice daily to flush the gaseous products of fermentation into

the gas absorption bottles and to maintain an anaerobic environment. The evolved carbon dioxide and nitrogen bases from fermentation were absorbed in 1.0 N KOH and 4% H_3BO_3, respectively. Each treatment at each harvest was fermented for 18 days at 32 C. Representative initial and fermented samples were collected, placed in polyethylene bags and immediately immersed in liquid nitrogen. The frozen samples were stored at -12 C until freeze-ground (Danley and Vetter, 1971). These samples were stored at -12 C until analyzed in the freeze-ground state.

A second group of 15 samples (100 g each) was taken at the same harvest time and assigned at random to three heat treatments (dried, microwaved and steam treated). Samples to be dried were dried at 100 C in a forced-draft oven to 12% moisture. Microwaved samples were treated for 6 min. in a Westinghouse microwave oven (Model KM520LXXI)[1]. Steam treated samples were prepared in a Wilmot Castle Autoclave (Model 32466)[1] for 7 min. at 126 C and under 1.2 kg/cm^2 pressure. These samples (dried, microwaved and steam treated) were ground in a Wiley Mill (2-mm screen) and stored in sealed glass jars at -12 C until analyzed.

Duplicate samples were chemically analyzed by the following procedures. Dry matter (DM) was determined on the freeze-ground samples by toluene distillation (DeWar and McDonald, 1961). Total carbohydrates (TCHO) were extracted by modifying enzyme procedures of MacRae and Armstrong (1968) and Auricchio et al. (1968). Corn samples (0.5 g freeze-ground, 0.4 g dry

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[1]Reference to this company or product name or other products used in this study does not imply approval or recommendation of the product by Iowa State University to the exclusion of others that may be suitable.

ground) were diluted with 15 ml distilled water, heated for 5 min. at 100

C and then autoclaved at 126 C for 60 min. to complete gelatinization of

the starch and eliminate microbial growth. After cooling, 10 ml acetate

buffer (0.2 M acetic acid-sodium acetate, pH 4.5) and 10 ml enzyme solu-

tion (0.2% Diazyme 160[2]) were added and the mixture incubated at 55 C for

40 hours.

Water soluble carbohydrates (SCHO) were extracted in a Waring Blender

by homogenizing a 2 g sample with 50 ml cold distilled water and filtered

through Whatman No. 40 filter paper. Ten ml of the filtered homogenate

were incubated at 39 C for 48 hr. with 10 ml acetate buffer and 10 ml

enzyme solution (0.5% Takadiatase[3]).

Corn samples (0.5 g) were diluted with 10 ml of distilled water and

allowed to stand at room temperature for 15 minutes. Ten ml of acetate

buffer and 10 ml of either α-amylase[4] or β-amylase[4] (0.2% solution, each)

were added and the resulting mixture incubated for 48 hr. at 39 C for the

extraction of α-amylase (α-SCHO) and β-amylase (β-SCHO) soluble carbo-

hydrates, respectively.

Ethanol soluble carbohydrates (ESCHO) were extracted by refluxing a

2 g sample with 50 ml hot (65 C) 80% ethanol on a Goldfisch Extractor

for 2 hours. Ten ml of the extract were incubated in the same manner as

the homogenized samples for determining SCHO.

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[2]Available from Miles Chemical Company, Clifton, New Jersey under the trade name "Takamine".

[3]Available from Parke, Davis and Company, Detroit, Michigan under the trade name "Taka-Diatase".

[4]Available from Sigma Chemical Company, St. Louis, Missouri.

After each incubation, the hydrolyzed solutions were filtered through Whatman No. 40 filter paper, diluted to 50 ml and stored in polyethylene bottles at 4 C until analyzed for reducing sugars by the modified potassium ferricyanide-potassium ferrocyanide oxidation-reduction method (Technicon Corporation, 1965).

The insoluble carbohydrate content (ISCHO) was calculated as the difference between the total and water soluble carbohydrate contents and is an estimate of the starch content (MacRae and Armstrong, 1968).

The volatile fatty acids in the freeze-ground ensiled corn were determined by a modification of the procedure described by Barnett (1954). An aqueous solution was obtained by shaking 1 ml of 5% mercuric chloride, 10 ml distilled water and 5 g sample for 4 hr at 4 C on a mechanical shaker. Then 1 ml of 25% metaphosphoric acid was added and after 10 min. the solution was centrifuged at 4 C for 10 min. at 12,000 g. The supernatant was stored at -12 C until analyzed for acetic, propionic and butyric acids as outlined by Baumgardt (1964).

Total nitrogen (TN) was determined on the Technicon Auto Analyzer using modifications of the procedure described by O'Neill and Webb (1970). Corn samples (1 g) were digested on a hot plate with 20 ml digestion mixture (82% sulfuric acid, 10% perchloric acid and 0.3% selenium dioxide). When digestion was complete the solutions were cooled, diluted to 50 ml and stored at 4 C until analyzed.

Absorbed carbon dioxide was determined volumetrically on triplicate aliquots of the potassium hydroxide solutions (A.O.A.C., 1965). Absorbed volatile nitrogen was determined on triplicate aliquots of the boric acid solutions by titration with 0.5 N HCl (Pierce and Haenisch, 1955).

The data were analyzed statistically by least squares analysis of variance for a completely randomized design followed by orthogonal comparisons of the treatment means as outlined by Snedecor and Cochran (1967).

Results and Discussion

Differences in DM, total nitrogen (TN) and gaseous losses due to artificially altering corn grain are shown in table 2.1. Artificially altering corn grain at different initial moisture contents resulted in significantly (P<.05) different DM, TN and gaseous losses. The lower DM, TN and gaseous losses from ensiling untreated corn harvested at 18% moisture indicate that little fermentation occurred. These losses, however, are not the result of seed respiration since under anaerobic conditions this effect is negligible (Milner and Geddes, 1945).

Differences in DM, TN and gaseous losses among methods of alteration were significant (P<.05). Similar significant differences occurred between ensiled corn and heat treated corn. Higher DM, TN and gaseous losses occurred when corn was ensiled at higher moisture contents. Increased DM and TN losses have been reported for corn grain stored at increasing moisture contents (Bottomley, Christensen and Geddes, 1950). For the most part, moisture content of ensiled corn was higher than that before ensiling, indicating that water is liberated during ensiling. Data reported by Nagel and Semeniuk (1947) attribute the increased water content to bacterial hydrolysis of the grain carbohydrates and proteins. The additional water may provide a more optimum environment for further organism development provided that enough moisture is available initially.

Dry matter losses of untreated-ensiled corn are similar to those reported by Burmeister, Hartman and Saul (1966). Higher volatile losses, especially CO_2, were associated with higher initial moisture content before ensiling. Data reported by Dexter (1966) indicate that moisture content and CO_2 loss are directly related and that below 20% moisture little CO_2 is produced anaerobically. A close relationship existed between total nitrogen loss and volatile nitrogen loss.

Dry matter, TN and volatile losses for reconstituted-ensiled corn were lower than those for untreated-ensiled corn when adequate moisture for fermentation was present. This difference is thought to be due to a reduction in substrate as a result of drying before reconstitution occurred. Regardless of initial moisture content, lower DM, TN and volatile losses occurred with the formic acid reconstituted corn. Similar results with formic acid treated grass silages reported by Barnett (1954) and Derbyshire, Waldo and Gordon (1971) indicate that formic acid lowers the initial pH and results in reduced proteolytic microbial activity and lower initial oxidative losses.

Losses in DM and TN for heat treated corn decreased with decreasing moisture content at harvest. At each harvest, losses were highest with microwaved corn followed by steamed and dried corn, respectively. There are no known reports that compare these methods of heat treatment, but the data suggest that the intense dry heat produced by the microwave process is more detrimental to the corn grain.

The effect of artificially altering corn grain on the TCHO, SCHO and ISCHO contents is shown in table 2.2. There was a significant (P<.05) increase in the carbohydrate fractions with decreasing moisture content at

harvest. Alteration of the corn had no significant (P>.05) effect on TCHO; however, differences in SCHO and ISCHO due to alteration were significant (P<.05). Compared to ensiled corn, heat treated corn had significantly (P<.05) lower SCHO and significantly (P<.05) higher ISCHO. Values for SCHO were significantly (P<.05) lower for reconstituted than for formic acid reconstituted corn. A similar significant difference was shown for the lower SCHO of microwaved compared to steamed corn. The lower SCHO of both reconstituted and microwaved corn were consistent with higher ISCHO values.

With the exception of untreated-ensiled corn harvested at 18% moisture and formic acid reconstituted corn, a reduction in SCHO occurred due to ensiling. Lower SCHO of ensiled corn are consistent with higher DM and volatile gaseous losses. This is in agreement with the data of Burmeister, Hartman and Saul (1966) which indicate that starch is resistant to normal microbial attack during the ensiling process. The data suggest that additional hydrolysis of starch occurred during the ensiling of untreated corn harvested at 18% moisture and ensiling of the formic acid reconstituted corn. This additional hydrolysis is possibly the result of differences in endogenous enzyme or microbial activity existing under these conditions of ensiling.

With forages, drying at high temperature significantly reduces the level of SCHO (Noller et al., 1966; Danley and Vetter, 1971). The data indicate that a similar reduction in SCHO results when corn grain is dried. The intense dry heat of the microwave process, particularly at the lower moisture contents, appears to have more of an effect on lowering

the SCHO. Steaming at higher initial moisture contents, however, had less of an effect than drying on lowering the SCHO.

Differences in the α-SCHO, β-SCHO and ESCHO contents due to artificially altering corn grain are shown in table 2.3. Alteration of corn at different initial moisture contents resulted in significant (P<.05) differences in the susceptibility of starch to both α-amylase and β-amylase enzymes. No significant (P>.05) differences in ESCHO existed due to decreasing moisture content at harvest. Alteration of corn grain resulted in significant (P<.05) differences in α-SCHO, β-SCHO and ESCHO. Similar significant differences existed between ensiled and heat treated corn.

Increased susceptibility of cereal grain starch to α-amylase and β-amylase has been used to determine the extent of gelatinization of artificially altered grain starch (Sullivan and Johnson, 1964; Osman et al., 1966; Liang et al., 1970). The data reported herein are in agreement with those reported by Hale (1971) in that no gelatinization of starch occurs during ensiling. Regardless of the method of ensiling and moisture content before ensiling, a reduction in α-SCHO and β-SCHO occurred. This effect was more pronounced with α-SCHO. The possibility does exist that this fraction is readily utilized by the developing microflora as an energy source. The significant (P<.05) reduction in α-SCHO of formic acid reconstituted corn compared to reconstituted corn is possibly the result of the enzyme being less active at the lower pH of formic acid reconstituted corn or the result of increased utilization by a more active microflora.

A significant (P<.05) increase in α-SCHO and β-SCHO occurred with steamed corn as compared to microwaved corn. Increased susceptibility of steamed sorghum starch to enzymatic attack has been reported (McNeill,

Potter and Riggs, 1970). The higher values of β-SCHO for steamed and microwaved corn compared to untreated corn indicate that gelatinization of corn starch occurred. The data suggest that at higher initial moisture content, flaking of steamed corn is not necessary for increased gelatinization of corn starch to occur. Data reported by Osman et al. (1966) indicate that steaming sorghum and barley at 1.4 kg/cm^2 for 1 min. without flaking decreased susceptibility of the starch to beta-amylolysis and that pressures of 4.2 or 5.6 kg/cm^2 were required to increase starch digestion over the untreated grain. The data reported herein are not in agreement with that reported by Liang et al. (1970) which showed a detrimental effect of increased processing time at high moisture contents; however, in this study a lower steaming pressure was used.

Drying reduced the susceptibility of the corn grain to α-amylase and β-amylase attack. Whistler, Goatley and Spencer (1959) reported that drying of corn starch results in the formation of cavities within the starch granule; however, at the surface of the granule a hardened shell is formed which decreases the rate of diffusion of chemicals into the granule. This exceeds the compensating effect of increased area due to cavitation and results in a reduced total granule reactivity.

The differences in ESCHO between microwaved corn and steamed corn were significant (P<.05). The reduction in ESCHO due to steaming is not in agreement with that reported by McNeill, Potter and Riggs (1970) with sorghum; however, the sorghum was reconstituted to 30% moisture before microwaved.

The volatile fatty acid content of reconstituted and formic acid reconstituted corn was significantly (P<.05) different than untreated

ensiled corn (table 2.4). Similar significant differences existed between reconstituted and formic acid reconstituted corn. Corn grain harvested at 16 and 22% moisture had higher acetate, propionate and butyrate contents than did either reconstituted or formic acid reconstituted corn. The lower acetate and propionate contents of corn harvested at 18% moisture indicate that little fermentation occurred. Acetate, propionate and butyrate contents were lower for reconstituted corn grain than for untreated-ensiled corn. Similar results have been reported by Byers et al. (1971). The lower acetate, propionate and butyrate contents of formic acid reconstituted corn grain are in agreement with the lower acetate and butyrate contents of formic acid treated grass silage (Waldo et al., 1969).

The data obtained in this study further indicate that the method of alteration significantly affects the dry matter and nitrogen losses and the carbohydrate fractions of corn grain and that the magnitude of these changes is related to the moisture content of the unaltered grain.

Summary

Changes in the carbohydrate fractions of artificially altered corn grain were determined on field corn harvested at 22, 18 and 16% moisture and altered by the following methods: untreated-ensiled, dried reconstituted-ensiled, formic acid reconstituted-ensiled, dried, microwaved and steamed.

Compared with ensiled corn, heat treated corn had significantly (P<.05) higher DM and TN losses. With adequate moisture, fermentation losses were lowest for formic acid reconstituted corn followed by reconstituted and untreated-ensiled corn grain, respectively. Losses in DM and TN for heat

altered corn decreased with decreasing moisture content at harvest and were lowest for dried corn followed by steamed and microwaved corn grain, respectively.

Alteration of corn grain had no significant (P>.05) effect on total carbohydrates; however, differences in water soluble carbohydrates (SCHO) and insoluble carbohydrates (ISCHO) due to alteration were significant (P<.05). Compared to ensiling, heat treated corn had significantly (P<.05) lower SCHO and significantly (P<.05) higher ISCHO. Values for SCHO were significantly (P<.05) lower for reconstituted than for formic acid reconstituted corn and for microwaved compared to steamed corn.

Artificially altering corn grain resulted in significant (P<.05) differences in α-amylase soluble carbohydrates (α-SCHO), β-amylase soluble carbohydrates (β-SCHO) and ethanol soluble carbohydrates (ESCHO) between ensiled and heat treated corn. Values for β-SCHO were highest for steamed corn followed by microwaved, untreated, dried, formic acid reconstituted, reconstituted and untreated-ensiled corn grain, respectively.

Acetate, propionate and butyrate contents of reconstituted and formic acid reconstituted corn were significantly (P<.05) different than those for untreated-ensiled grain. Significant (P<.05) differences also existed between reconstituted and formic acid reconstituted corn. Acetate, propionate and butyrate contents were lower for formic acid reconstituted corn grain than for reconstituted and untreated-ensiled corn, respectively.

TABLE 2.1. DRY MATTER (DM), TOTAL NITROGEN (TN) AND GASEOUS LOSSES OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

| Item | Method of alteration[b] | | | | | |
	Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
			22% moisture[c]			
Initial DM, %	77.9	74.9	72.9	77.9	77.9	77.9
Final DM, %	76.0	75.0	73.4	86.5	88.5	75.2
DM loss, %[d,e,f]	3.50	2.27	1.72	3.93	5.68	4.34
Initial TN, mg/g	16.0	16.0	15.5	16.0	16.0	16.0
Final TN, mg/g	16.2	16.0	15.6	16.3	16.0	15.9
TN loss, %[d,e,f]	2.56	2.25	1.27	2.32	5.63	4.87
CO_2 loss, mg/g[d,e]	5.60	4.20	3.80	--	--	--
Volatile N, μg/g[d,e]	384	324	181	--	--	--
			18% moisture[c]			
Initial DM, %	82.0	77.3	72.7	82.0	82.0	82.0
Final DM, %	81.6	76.9	72.1	86.6	87.2	75.4
DM loss, %[d,e,f]	1.38	2.00	1.62	2.83	4.49	3.60
Initial TN, mg/g	16.0	15.8	15.6	16.0	16.0	16.0
Final TN, mg/g	16.1	15.9	15.6	16.2	16.0	16.1
TN loss, %[d,e,f]	0.76	1.38	1.01	1.32	3.92	2.46
CO_2 loss, mg/g[d,e]	3.40	4.00	3.00	--	--	--
Volatile N, μg/g[d,e]	132	238	115	--	--	--

	16% moisture[c]					
Initial DM, %	74.5	72.9	73.0	84.5	84.5	84.5
Final DM, %	72.7	72.7	72.5	86.9	86.7	75.5
DM loss, %[d,e,f]	3.79	1.82	1.33	2.21	3.70	3.20
Initial TN, mg/g	16.0	16.0	15.8	16.0	16.0	16.0
Final TN, mg/g	16.3	16.0	15.8	16.0	16.1	16.1
TN loss, %[d,e,f]	1.75	1.98	1.39	0.86	1.87	1.29
CO_2 loss, mg/g[d,e]	5.90	3.90	3.40	--	--	--
Volatile N, μg/g[d,e]	272	308	201	--	--	--

[a]Values expressed on dry matter basis.

[b]Untreated-ensiled, dried reconstituted-ensiled, formic acid reconstituted-ensiled, dried, microwaved and steamed, respectively.

[c]Original moisture content at harvest.

[d]Significant ($P<.05$) differences due to decreasing moisture content at harvest.

[e]Significant ($P<.05$) difference among treatments.

[f]Significant ($P<.05$) difference between ensiled and heat treated corn.

TABLE 2.2. TOTAL, WATER SOLUBLE AND INSOLUBLE CARBOHYDRATE CONTENTS OF ARTIFICIALLY
ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Original moisture level, %	Unaltered	Method of alteration					
		Ensiled			Heat treated		
		Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
Total carbohydrates, mg/g[b]							
22	717.5	720.8	731.3	739.4	748.5	753.6	707.1
18	757.8	747.6	748.2	765.1	774.7	785.6	746.0
16	774.2	752.4	754.7	772.1	786.4	795.4	760.5
Water soluble carbohydrates, %[b,c,d,e]							
22	4.7	3.8	2.6	5.8	3.4	3.4	4.8
18	4.8	6.4	3.4	6.6	4.0	3.1	4.2
16	5.7	5.0	4.1	7.1	5.2	3.2	3.8
Insoluble carbohydrates, mg/g[b,c]							
22	638.5	693.5	712.0	696.7	723.0	728.1	673.3
18	721.5	699.4	722.7	714.2	743.7	761.2	714.2
16	729.8	715.2	723.9	717.6	745.7	769.6	731.2

[a]Values expressed on dry matter basis. Water soluble carbohydrates expressed as percent of the total carbohydrate content on dry matter basis.

[b]Significant (P<.05) differences due to decreasing moisture content at harvest.

[c]Values for artificially altered corn significantly (P<.05) different than those for untreated corn.

[d]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[e]Values for reconstituted corn significantly (P<.05) different than those for formic acid reconstituted corn. Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 2.3. α-AMYLASE, β-AMYLASE AND ETHANOL SOLUBLE CARBOHYDRATE CONTENTS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Original moisture level, %	Unaltered	Ensiled			Heat treated		
		Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
α-amylase soluble carbohydrates, %[b,c,d,e,f]							
22	20.6	8.8	7.8	4.4	15.0	20.4	25.3
18	19.3	9.4	8.1	6.2	15.1	19.6	24.2
16	18.2	9.7	8.2	6.3	15.1	19.9	24.1
β-amylase soluble carbohydrates, %[b,c,d,f]							
22	13.1	11.3	11.7	12.3	12.7	33.6	42.3
18	13.2	11.0	11.8	12.6	12.8	26.4	28.8
16	13.3	12.4	12.5	12.7	13.3	17.0	24.7
Ethanol soluble carbohydrates, %[c,d,f]							
22	7.8	7.6	8.0	8.5	9.8	9.0	7.3
18	7.1	6.3	6.9	8.3	9.3	8.9	7.2
16	7.1	7.8	8.0	8.6	9.3	9.2	7.5

[a]Values expressed as percent of total carbohydrate content on dry matter basis.

[b]Significant (P<.05) differences due to decreasing moisture content at harvest.

[c]Values for artificially altered corn significantly (P<.05) different than those for untreated corn.

[d]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[e]Values for reconstituted corn significantly (P<.05) different than those for formic acid reconstituted corn.

[f]Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 2.4. ACETATE, PROPIONATE AND BUTYRATE CONTENTS OF ENSILED
CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Moisture level at harvest, %	Method of alteration[b]		
	Ensiled	Reconstituted	Formic acid reconstituted
Acetate, mg/g[c,d]			
22	1.38	1.32	1.08
18	0.84	1.26	1.05
16	1.44	1.20	1.02
Propionate, mg/g[c,d]			
22	0.64	0.65	0.54
18	0.37	0.60	0.54
16	0.70	0.62	0.55
Butyrate, mg/g[c,d]			
22	0.86	0.79	0.79
18	0.87	0.82	0.78
16	0.97	0.85	0.74

[a]Values expressed on dry matter basis.

[b]Untreated-ensiled, dried reconstituted-ensiled, and formic acid reconstituted-ensiled, respectively.

[c]Values for untreated-ensiled corn significantly ($P<.05$) different than those for treated-ensiled corn.

[d]Values for reconstituted-ensiled corn significantly ($P<.05$) different than those for formic acid reconstituted corn.

SECTION III:

CHANGES IN THE NITROGEN FRACTIONS OF

ARTIFICIALLY ALTERED CORN GRAIN HARVESTED

AT THREE MOISTURE LEVELS

Introduction

There is a paucity of information in the literature concerning the extent of changes in protein solubility due to the alteration of corn grain. Data reported by McGuire and Earle (1958) indicate that the solubility of protein decreases with increased drying temperatures ranging from 49 to 93 C. Associated with the uptake of water, insoluble protein in the endosperm drastically decreases during germination (Ingle, Beevers and Hageman, 1964). A similar process is thought to occur during the reconstitution of cereal grains (Riggs, 1969). McNeill, Potter and Riggs (1970) suggest that the microwaving process could denature the poorly soluble endosperm proteins of sorghum to make them even less soluble. On the other hand, steaming followed by proper flaking is thought to increase protein solubility (Hale, 1971).

In view of the limited information on the changes in the solubility of corn grain protein, this study was conducted to determine the effect of artificially altering corn grain on the changes in the nitrogen fractions and to compare the effects of initial moisture content on the extent of these changes.

Materials and Methods

Corn of a single variety (Pioneer 3570) was harvested at 22, 18 and 16% moisture from a common experimental plot. At each harvest, representative samples were taken, assigned at random to three fermentation treatments (untreated-ensiled, dried reconstituted-ensiled and formic acid reconstituted-ensiled) and ensiled as described by Danley and Vetter

(1972b). Representative samples of the fresh and ensiled high moisture

corn grain were freeze-ground (Danley and Vetter, 1971) and stored at

-12 C until analyzed. A second group of samples was taken at each harvest,

assigned at random to three heat treatments (dried, microwaved and steam

treated) and prepared as described by Danley and Vetter (1972b). These

samples were ground in a Wiley Mill (2-mm screen) and stored in sealed

glass jars at -12 C until analyzed.

Duplicate samples were chemically analyzed by the following proce-

dures. Dry matter (DM) was determined on the freeze-ground samples by

toluene distillation (DeWar and McDonald, 1961). Total nitrogen (TN)

was determined on the Technicon Auto Analyzer using modifications of the

procedure described by O'Neill and Webb (1970). Corn samples (1 g) were

digested on a hot plate with 20 ml digestion mixture (82% sulfuric acid,

10% perchloric acid and 0.3% selenium dioxide). When digestion was

complete the solutions were cooled, diluted to 50 ml with distilled water

and stored at 4 C until analyzed.

Water soluble nitrogen (WSN), sodium chloride soluble nitrogen (SCSN),

ethanol soluble nitrogen (ESN) and sodium hydroxide soluble nitrogen (SHSN)

were extracted by modifications of the successive solvent extraction

technique described by Skoch et al. (1970). Distilled water (25 ml) was

added to 0.5 g sample. The resulting suspension was shaken horizontally

for 2 hr. at room temperature, centrifuged at 3000 g for 15 min. and the

supernatant decanted. The insoluble residue was washed with 20 ml

distilled water, shaken for 15 min., centrifuged at 3000 g for 15 min.

and the supernatant decanted. The two supernatants were combined,

diluted to 50 ml with distilled water and stored at 4 C. Similarly, the

remaining insoluble residue was successively extracted with 5% sodium chloride, ethanol (80% ethanol, 18.8% water and 0.2% sodium acetate) and 0.2% sodium hydroxide. The insoluble residue remaining after each extraction was frozen (-12 C overnight) and thawed to increase nitrogen solubility. Ten ml aliquots of the diluted supernatant solutions were digested on a hot plate with 10 ml of the digestion mixture used for determining TN, cooled, diluted to 50 ml with distilled water and stored at 4 C until analyzed for ammonia colorimetrically by the phenol-hypochlorite procedure described by O'Neill and Webb (1970).

Total insoluble nitrogen (TISN) was estimated as the difference between TN and total soluble nitrogen obtained by successive solvent extractions.

Hot ethanol soluble nitrogen (HESN) was extracted from a 2 g corn sample by the technique described by Sprague and Breniman (1969). Extracts were diluted to 50 ml with 80% ethanol and stored at 4 C. The ethanol in a 20 ml aliquot of the extract was removed by evaporation. The remaining ethanol soluble nitrogen was digested with 10 ml digestion mixture and analyzed for ammonia by the procedure described for super-natants obtained by successive solvent extraction. Ammonia production from the HESN extract was determined on a second aliquot (20 ml). After removal of the ethanol by evaporation, the remaining ethanol soluble nitrogen was hydrolyzed by refluxing with 10 ml 6 N HCl for 60 minutes. The hydrolysate was cooled, diluted to 25 ml and analyzed for ammonia (Technicon Corporation, 1960).

Acid pepsin soluble nitrogen (PSN) was extracted by the technique described by Terry and Tilley (1964). The resulting suspension was

centrifuged at 3000 g for 15 min., diluted to 50 ml with distilled water and stored at 4 C until digested and analyzed for ammonia by the procedure used for supernatants obtained by successive solvent extraction.

Soluble ammonia nitrogen (NH_3-N) was determined on the WSN extract as described by Technicon Corporation (1960). Also from the WSN extract, α-amino nitrogen (NH_2-N) was determined colorimetrically on the Technicon Auto Analyzer by the ninhydrin procedure as modified by Fisher, Bunting and Rosenberg (1963).

The data were analyzed statistically by least squares analysis of variance for a completely randomized design followed by orthogonal comparisons of treatment means as outlined by Snedecor and Cochran (1967).

Results and Discussion

The DM and TN contents of artificially altered corn grain are shown in table 3.1. The DM contents of the ensiled corn grain approached the expected values. However, due to variation in the rate of water uptake by intact corn grain of different moisture contents, slight over and under reconstitution occurred. Differences in DM among methods of ensiling are primarily the result of variation in the amount of water absorbed by the intact grain before ensiling since little change in DM occurred during fermentation (Danley and Vetter, 1972b). The data indicate that drying before reconstitution reduces the water absorption capacity of the intact kernel. Also, there appears to be a limit to the amount of water that can be absorbed by the corn during steaming.

Although the TN content of formic acid reconstituted corn was

consistently lower, differences in TN due to initial moisture content and method of alteration were not significant (P>.05).

The WSN, SCSN, ESN, SHSN and TSN contents from the successive extraction of artificially altered corn grain are shown in table 3.2. The data show that the greatest differences occurred with the WSN and SCSN fractions. Regardless of the nitrogen fraction, significant (P<.05) differences in nitrogen solubility occurred with decreasing moisture content at harvest. Compared to ensiling, heat treated corn had a significantly (P<.05) lower TSN content which is the result of significantly (P<.05) lower WSN, SCSN, ESN and SHSN fractions of the heat treated corn. Nagel and Semeniuk (1947) suggest that the increase in water soluble nitrogen with a corresponding decrease in insoluble nitrogen in corn grain stored at high moisture contents reflects metabolic activities of the microorganisms present. Microbial growth is enhanced under conditions of high moisture, adequate temperature and low aeration (Burmeister, Hartman and Saul, 1966; Burmeister and Hartman, 1966). Substantial increases in WSN, SCSN and TSN with corresponding decreases in TISN occurred during ensiling except for corn harvested at 18% moisture and ensiled since little fermentation occurred at this moisture content (Danley and Vetter, 1972b).

Riggs (1969) suggests that the processes occurring during reconstitution are similar to those that occur during germination. Germination is characterized by little loss of total nitrogen, rapid increase in soluble nitrogen and a decrease in insoluble nitrogen as the result of increased endogenous enzyme activity (Ingle, Beevers and Hageman, 1964). The data indicate that during reconstitution considerable increases in TSN occur which parallel decreases in TISN. Histological studies with reconstituted

sorghum show a disorganization of the protein matrix (Hale, 1971). If disorganization or disruption of the protein matrix occurs during reconstitution, relatively large increases in ethanol soluble nitrogen from the release or hydrolysis of zein located within the protein matrix should occur. The small increases in ESN relative to the increased TSN of reconstituted corn does not indicate that any appreciable physical disruption of the protein matrix occurred. It is possible that the increased WSN and SCSN of reconstituted corn originates from the hydrolysis of zein which has been released from the protein matrix during the reconstitution processes. Similar increases in soluble protein found in the endosperm of germinating corn are thought to originate from the hydrolysis of zein located within the protein matrix (Duvick, 1961).

The WSN, SCSN and TSN fractions of reconstituted corn were significantly (P<.05) lower and TISN was significantly (P<.05) higher than for formic acid reconstituted corn. The data indicate that increased hydrolysis of the insoluble protein occurred during ensiling of formic acid reconstituted corn as the result of increased endogenous enzyme or microbial activity. The possibility does exist that drying before reconstitution reduces endogenous enzyme activity or decreases the solubility of readily available substrates. Thus, the overall effect of reconstitution is reduced.

The reduced TSN content of dried corn grain is in agreement with data reported by McGuire and Earle (1958). The lower ESN content of the dried corn in this study is the result of slight modifications in the extraction of ESN. The large differences in TSN between ensiled and dried corn

indicated that in addition to the WSN fraction other nitrogen fractions
are also denatured during drying.

Water soluble nitrogen, SCSN, ESN, SHSN and TSN were significantly
(P<.05) lower whereas TISN was significantly (P<.05) higher for microwaved
corn compared to steamed corn (table 3.2). There are no reported
comparisons of the effects of drying, microwaving and steaming on nitrogen
solubility; however, the data indicate that the intense dry heat of the
microwave process results in more denaturation of the protein than either
drying or steaming. Similar decreases in soluble nitrogen have been
reported for dry heat expanded (popped) grains (Walker et al., 1970).
On the other hand, data reported herein suggest that steaming has a
positive effect on nitrogen solubility compared to drying and microwaving.
This is possibly due to hydration of the protein matrix from the moist heat
treatment during steaming (Hale, 1971) or disruption of the starch granule
which permits greater access to the ethanol soluble protein bodies and to
the insoluble matrix protein (Nielsen et al., 1970). The data are not in
agreement with those of McNeill, Potter and Riggs (1970) in that increased
protein solubility is not generally associated with unflaked, steamed
grains; however, the solubility of unaltered sorghum grain is lower than
that of unaltered corn grain (Skoch et al., 1970).

The effects of artificially altering corn grain on the NH_3-N, HESN and
NH_3 production are shown in table 3.3. Differences in NH_3-N due to
decreasing moisture content at harvest approached significance (P<.05)
which was the result of the higher NH_3-N of ensiled corn harvested at 16%
moisture. Compared with ensiled corn, heat treated corn had a significantly
(P<.05) lower NH_3-N content which indicates that less hydrolysis of the

soluble nitrogen occurred with heat treated corn. The lower NH_3-N of corn grain harvested at 18% moisture and ensiled suggests that little fermentation occurred because moisture was inadequate for the development of an active microflora. Formic acid reconstituted corn grain had significantly ($P<.05$) lower NH_3-N than reconstituted corn. Similar results have been reported for formic acid treated grass silages (Waldo et al., 1969).

Differences in HESN due to corn grain alterations at different initial moisture contents were significant ($P<.05$). Ensiled corn had significantly ($P<.05$) higher HESN content than heat treated corn. Similar increases in HESN have been reported by Sprague and Breniman (1969). As expected, HESN was closely related to the TSN content obtained by successive extraction of corn proteins. This fraction is not considered to be representative of prolamine nitrogen since lack of consecutive solvent extraction would allow overlap of the proteins in a given solubility class (Skoch et al., 1970). HESN of reconstituted corn was significantly ($P<.05$) lower than that for formic acid reconstituted corn. Similar significant differences existed for the lower HESN of microwaved compared with steamed corn. This is consistent with changes in TSN. Since prolamines yield large amounts of ammonia upon hydrolysis, an attempt was made to determine what effect increased HESN had on ammonia release. Ammonia production from the hydrolysis of the HESN fractions from ensiled corn was significantly ($P<.05$) lower than from heat treated corn. The lower ammonia production from ensiled corn is likely the result of higher initial HESN contents rather than a slower rate of ammonia release.

Artificially altering corn grain at different initial moisture contents resulted in significant ($P<.05$) differences in PSN (table 3.4).

Values for PSN were significantly (P<.05) higher for ensiled corn than for heat treated corn. Similar significant differences existed for the lower PSN of microwaved corn compared to steamed corn. For the most part, values for PSN were similar to those for TSN; however, PSN tended to underestimate nitrogen solubility of ensiled corn and overestimate nitrogen solubility of microwaved corn. The reasons for these differences cannot be explained from the results of this study.

As shown in table 3.4, significant (P<.05) differences in NH_2-N occurred when corn grain was artificially alterated at different initial moisture contents. The significant (P<.05) increase in NH_2-N of ensiled corn suggests that considerable hydrolysis of insoluble protein occurs during fermentation. The low NH_2-N content of corn grain harvested at 18% moisture and ensiled further indicates that little fermentation occurred at this moisture level. Differences in NH_2-N between reconstituted and formic acid reconstituted corn and between microwaved and steamed corn were significant (P<.05).

Danley and Vetter (1972b) previously reported that the method of alteration affects the carbohydrate fractions of corn grain. It appears that alteration of the corn grain results in opposing effects on carbohydrate and nitrogen solubility. Contrasted to drying which decreases both carbohydrate and nitrogen solubility, ensiling and reconstitution does not appreciably increase carbohydrate solubility but does increase the amount of soluble nitrogen. On the other hand, the carbohydrates of microwaved and steamed corn are more susceptible to enzymatic attack but in the case of microwaved corn, large decreases in nitrogen solubility occur.

The data obtained in this study indicate that the method of alteration of corn grain significantly affects nitrogen solubility and that these changes are related to the moisture content of the unaltered grain.

Summary

Changes in nitrogen solubility were determined on field corn harvested at 22, 18 and 16% moisture and altered by the following methods: untreated-ensiled, dried reconstituted-ensiled, formic acid reconstituted-ensiled, dried, microwaved and steamed. Differences in the total nitrogen (TN) content of artificially altered corn grain were not significantly ($P > .05$) affected by method of alteration or initial moisture content.

Water soluble (WSN), sodium chloride (SCSN), ethanol, sodium hydroxide and total soluble nitrogen (TSN) fractions of artificially altered corn grain were determined by successive extractions. Regardless of extraction, significant ($P < .05$) differences in nitrogen solubility occurred with decreasing moisture content at harvest. The WSN and SCSN fractions were affected to a greater extent by method of alteration. Values for TSN were lowest for microwaved corn followed by dried, steamed, untreated, ensiled, reconstituted and formic acid reconstituted corn, respectively. Associated with increased TSN was a corresponding decrease in insoluble nitrogen. For the most part, significant ($P < .05$) differences in nitrogen solubility occurred between reconstituted and formic acid reconstituted corn and between microwaved and steamed corn.

Ensiled corn had significantly ($P < .05$) higher levels of ammonia nitrogen than heat treated corn. Values for ammonia nitrogen were significantly ($P < .05$) higher for reconstituted than formic acid

reconstituted corn. Significant (P<.05) differences in α-amino nitrogen (NH_2-N) occurred when corn grain was artificially altered at different initial moisture contents. Ensiling resulted in significant (P<.05) increases in NH_2-N compared to heat treating corn grain. Similar significant differences existed between reconstituted and formic acid reconstituted corn and between microwaved and steamed corn.

Changes in the hot ethanol soluble nitrogen (HESN) and acid-pepsin soluble nitrogen (PSN) due to artificially altering corn grain were determined. Similar relationships between HESN and PSN occurred; however, HESN was more closely related to TSN than was PSN.

TABLE 3.1. DRY MATTER AND TOTAL NITROGEN CONTENTS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

| Original moisture level, % | Unaltered | Method of alteration | | | | | |
| | | Ensiled | | | Heat treated | | |
		Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
Dry matter, $\%$[b,c]							
22	77.9	76.0	75.0	73.4	86.5	88.5	75.2
18	82.0	81.6	76.9	72.1	86.6	87.2	75.4
16	84.5	72.7	72.7	72.5	86.9	86.7	75.5
Total nitrogen, mg/g							
22	16.0	16.2	16.0	15.6	16.3	16.0	15.9
18	16.0	16.1	15.9	15.6	16.2	16.0	16.1
16	16.0	16.3	16.0	15.8	16.0	16.1	16.1

[a]Values expressed on dry matter basis.

[b]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[c]Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 3.2. WATER, SODIUM CHLORIDE, ETHANOL, SODIUM HYDROXIDE AND TOTAL SOLUBLE AND INSOLUBLE NITROGEN CONTENTS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Original moisture level, %	Unaltered	Method of alteration					
		Ensiled			Heat treated		
		Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
Water soluble nitrogen, %[b,c,d,e,f]							
22	10.2	14.2	16.2	19.6	8.7	7.6	9.5
18	9.2	11.4	15.1	18.5	7.9	6.6	8.8
16	8.7	13.4	13.0	15.2	7.1	6.0	8.1
Sodium chloride soluble nitrogen, %[b,d,e,f]							
22	9.4	12.0	14.2	16.6	8.7	7.7	8.3
18	10.6	11.4	13.2	15.1	9.8	8.8	9.7
16	12.4	11.0	13.4	14.7	10.7	9.9	11.4
Ethanol soluble nitrogen, %[b,d,f]							
22	21.0	23.5	24.6	25.6	18.3	10.9	20.6
18	22.0	22.2	23.1	25.0	20.3	12.9	21.8
16	23.2	23.2	23.5	24.3	21.2	15.7	22.8
Sodium hydroxide soluble nitrogen, %[b,d,f]							
22	22.6	24.4	26.8	28.4	19.9	13.0	20.8
18	25.0	23.4	26.3	27.6	21.4	15.3	22.4
16	26.6	26.1	25.3	27.3	22.2	17.1	24.2

Total soluble nitrogen, $\%^{b,d,e,f}$

22	63.3	74.1	81.8	90.2	55.5	39.2	59.1
18	66.7	68.4	77.7	86.2	59.3	43.7	62.7
16	71.2	73.7	75.2	81.5	61.2	48.7	66.5

Total insoluble nitrogen, $\%^{b,d,e,f}$

22	36.7	25.9	18.2	9.8	44.5	60.8	40.9
18	33.3	31.6	22.3	13.8	40.7	56.3	37.3
16	28.8	26.3	24.8	18.5	38.8	51.3	33.5

[a]Values expressed as percent of total nitrogen content on dry matter basis.

[b]Significant (P<.05) differences due to decreasing moisture content at harvest.

[c]Values for artificially altered corn significantly (P<.05) different than those for untreated corn.

[d]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[e]Values for reconstituted corn significantly (P<.05) different than those for formic acid reconstituted corn.

[f]Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 3.3. AMMONIA AND HOT ETHANOL SOLUBLE NITROGEN CONTENTS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

| Original moisture level, % | Unaltered | Method of alteration | | | | | |
| | | Ensiled | | | Heat treated | | |
		Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
		Ammonia nitrogen, %[c,d,e]					
22	51.2	61.4	60.1	42.4	51.4	51.2	48.7
18	51.4	44.8	66.9	45.1	55.7	53.9	51.8
16	51.4	85.4	83.4	62.8	57.4	52.1	50.8
		Hot ethanol soluble nitrogen, %[b,c,d,e,f]					
22	42.8	53.5	51.5	57.9	39.9	26.4	34.4
18	35.6	50.3	57.5	61.5	35.9	26.0	30.6
16	32.8	60.1	62.2	70.0	30.6	22.1	22.9
		Ammonia production, %[d]					
22	22.0	16.9	17.7	18.4	19.5	21.6	30.2
18	25.4	18.2	18.2	17.2	23.7	24.4	31.0
16	25.1	16.8	17.5	17.2	29.6	30.4	34.3

[a]Ammonia nitrogen expressed as percent of water soluble nitrogen on dry matter basis. Values for soluble nitrogen expressed as percent of total nitrogen on dry matter basis. Values for ammonia production expressed as percent of soluble nitrogen.

[b]Significant (P<.05) differences due to decreasing moisture content at harvest.

[c]Values for artificially altered corn significantly (P<.05) different than those for untreated corn.

[d]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[e]Values for reconstituted corn significantly (P<.05) different than those for formic acid reconstituted corn.

[f]Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 3.4. PEPSIN AND α-AMINO SOLUBLE NITROGEN CONTENTS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Original moisture level, %	Unaltered	Method of alteration			Heat treated		
		Ensiled					
		Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
Pepsin soluble nitrogen, %[b,d,f]							
22	59.5	64.3	68.3	75.4	51.1	54.6	60.9
18	57.2	59.4	65.0	70.4	52.8	51.0	63.1
16	56.4	62.6	63.0	66.4	57.4	50.4	67.6
α-amino nitrogen, mm[b,c,d,e,f]							
22	3.2	58.4	60.1	63.9	3.4	1.3	1.4
18	3.6	20.7	85.2	85.2	3.4	1.4	1.5
16	5.4	63.9	94.2	123.9	5.1	1.4	1.6

[a]α-amino nitrogen expressed on dry matter basis. Values for pepsin soluble nitrogen expressed as percent of total nitrogen content on dry matter basis.

[b]Significant (P<.05) differences due to decreasing moisture content at harvest.

[c]Values for artificially altered corn significantly (P<.05) different than those for untreated corn.

[d]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[e]Values for reconstituted corn significantly (P<.05) different than those for formic acid reconstituted corn.

[f]Values for microwaved corn significantly (P<.05) different than those for steamed corn.

74

SECTION IV:

THE _IN_ _VITRO_ UTILIZATION OF THE CARBOHYDRATE AND

NITROGEN FRACTIONS OF ARTIFICIALLY ALTERED CORN GRAIN

HARVESTED AT THREE MOISTURE LEVELS

Introduction

In previous reports (Danley and Vetter, 1972b,c), it was shown that the solubility of the carbohydrate and nitrogen fractions of corn grain is significantly affected by processing methods which alter the kernel composition and structure. Increased digestibility of nonprotein organic matter reported by Buchanan-Smith, Totusek and Tillman (1968) and Husted et al. (1968) suggests that alteration of sorghum grain improves the availability or utilization of the starch portion. Results reported by McNeill, Potter and Riggs (1971) and Potter, McNeill and Riggs (1971) indicate that starch and protein digestion in the rumen were improved in steers fed reconstituted and steam-flaked sorghum grain. With dry-ground and microwaved grain ruminal digestion of both starch and protein was impaired. Similar digestibility data have not been reported for corn grain, and little is known concerning the effect of initial moisture content on the carbohydrate and nitrogen utilization of artificially altered corn grain.

The objectives of this study were to determine the effect of artificially altering corn grain on the in vitro carbohydrate and nitrogen utilization and to compare the responses at different initial moisture contents.

Materials and Methods

Corn of a single variety (Pioneer 3570) was harvested at 22, 18 and 16% moisture from a common experimental plot. At each harvest, representative samples of untreated-ensiled, dried reconstituted-ensiled,

formic acid reconstituted-ensiled, dried, microwaved and steamed corn grain were prepared as previously described by Danley and Vetter (1972b).

Duplicate samples of the artificially altered corn grain were chemically analyzed by the following procedures. In vitro digestible dry matter (IDDM) was determined after 12 hr. by the two-stage in vitro digestion procedure of Tilley and Terry (1963). The IDDM of corn harvested at 22% moisture and artificially altered was also determined at 24 and 48 hours. Lengths of the pepsin digestion were adjusted to correspond with the length of digestion with the buffered rumen fluid.

The in vitro digestion procedure of Tilley and Terry (1963) was further modified to estimate the extent of ruminal utilization of the carbohydrate and nitrogen contents of artificially altered corn grain. Corn samples (0.5 g) were incubated anaerobically with 40 ml rumen fluid-buffer mixture (1 part rumen fluid:4 parts buffer) for 12 hours. Corn harvested at 22% moisture and artificially altered was also incubated with the rumen fluid-buffer for 24 hours. Rumen fluid was collected from a fistulated Jersey cow maintained on an alfalfa hay diet supplemented with 1.5 kg corn daily. The fluid was strained through four layers of cheese-cloth before being added to the buffer (McDougall, 1948). After incubation, microbial activity was checked by the addition of 1 ml mercuric chloride (5% solution). Samples were then stored at 4 C, centrifuged at 3000 g for 15 min. and the supernatant decanted. The insoluble residue was washed with 10 ml distilled water, centrifuged at 3000 g for 15 min. and the supernatant decanted. The two supernatants were combined, diluted to 50 ml with distilled water and stored at 4 C, and the remaining insoluble residue was sealed and stored at -12 C until analyzed.

Aliquots of the supernatants were analyzed for total soluble nitrogen (TSN) and ammonia nitrogen (NH_3-N) as previously described by Danley and Vetter (1972c). Volatile fatty acid production was determined on deproteinated aliquots by the procedure of Baumgardt (1964).

The insoluble residues were analyzed for residual total carbohydrates (TCHO), α-amylase soluble carbohydrates (α-SCHO) and β-amylase soluble carbohydrates (β-SCHO) as previously described by Danley and Vetter (1972b). Values for digestible total carbohydrates (DTCHO) were estimated as the difference between the TCHO content of the altered grain before incubation and residual TCHO and expressed as a percent of the initial TCHO content. Similarly, digestible α-amylase soluble carbohydrates (α-DSCHO) and digestible β-amylase soluble carbohydrates (β-DSCHO) were estimated by difference and expressed either as percentages of the initial α-SCHO and β-SCHO contents or as percentages of the residual TCHO.

The total insoluble nitrogen (TISN) content of the insoluble residues was determined by two successive digestions with pepsin as described by Danley and Vetter (1972c). Residual ethanol soluble nitrogen (ESN) and residual sodium hydroxide soluble nitrogen (SHSN) contents of the insoluble residues were extracted by the successive solvent extraction technique described by Skoch et al. (1970) as modified by Danley and Vetter (1972c). Values for digestible ethanol soluble nitrogen (DESN) were estimated as the difference between the ESN content of the altered grain before incubation and residual ESN and expressed as percent of the initial ESN. Residual nitrogen was calculated as the difference between TISN and the sum of residual ESN and SHSN and expressed as percent of TISN.

The data were analyzed statistically by least squares analysis of variance for a completely randomized design followed by orthogonal comparisons of treatment means as outlined by Snedecor and Cochran (1967).

Results

Carbohydrate utilization

The effects of artificially altering corn grain on the IDDM and DTCHO are shown in table 4.1. Significant (P<.05) increases in IDDM with increased incubation time occurred with corn harvested at 22% moisture, and differences that existed at 12 hr. remained essentially unchanged at 24 and 48 hours. Similar significant increases in DTCHO occurred with increased incubation time.

With the 12 hr. incubation, differences in IDDM and DTCHO due to artificially altering corn grain harvested at different moisture contents were not significant (P>.05). Compared with ensiled corn, heat treated corn had significantly (P<.05) lower IDDM and DTCHO. IDDM and DTCHO were significantly (P<.05) lower for reconstituted than for formic acid reconstituted corn. Similar significant differences were shown for the lower IDDM and DTCHO of microwaved compared to steamed corn.

Residual α-SCHO and β-SCHO increased significantly (P<.05) with increased incubation time (table 4.2). α-DSCHO and β-DSCHO decreased with increased incubation time but the differences were not significant (P>.05).

With the 12 hr. incubation, no significant (P>.05) differences in α-DSCHO or β-DSCHO existed. However, values for α-DSCHO tended to increase as IDDM increased. An inverse relationship was shown between β-DSCHO and

IDDM. Differences in residual α-SCHO and β-SCHO due to harvesting corn at different moisture contents were significant (P<.05). Values for residual α-SCHO tended to decrease while those for residual β-SCHO tended to increase as IDDM increased except for microwaved and steamed corn where the inverse occurred. Heat treated corn had significantly (P<.05) higher residual α-SCHO and β-SCHO than ensiled corn. Significant (P<.05) differences in residual α-SCHO and β-SCHO existed between reconstituted and formic acid reconstituted corn and between microwaved and steamed corn.

The effect of artificial alteration of corn grain on in vitro volatile fatty acid (VFA) production is shown in table 4.3. Compared with the 12 hr. incubation, significant (P<.05) increases in VFA production occurred with the 24 hr. incubation. Since acetate production remained high in relation to propionate production, higher acetate:propionate ratios were found at the 24 hr. incubation.

With the 12 hr. incubation differences in propionate, butyrate and valerate production due to alteration of corn grain harvested at different moisture contents were significant (P<.05). Acetate production for heat treated corn was significantly (P<.05) lower and propionate production significantly (P<.05) higher than for ensiled corn. Similar significantly lower rates of VFA production existed for reconstituted and microwaved corn compared with formic acid reconstituted and steamed corn, respectively.

Nitrogen utilization

The in vitro TSN, NH_3-N and TISN contents of artificially altered corn grain are shown in table 4.4. With the 24 hr. incubation, significant (P<.05) increases in TSN were inversely related to the significant (P<.05)

decrease in TISN. Although NH_3-N tended to be lower at the 24 hr. incubation, the differences were not significant (P>.05).

Significant (P<.05) differences in TSN contents occurred with corn altered at different initial moisture contents. Higher TSN contents were associated with increased IDDM. NH_3-N tended to be higher with altered corn grain of low IDDM; however, differences between ensiled and heat treated corn were not significant (P>.05). TSN was significantly (P<.05) lower and NH_3-N and TISN were significantly (P<.05) higher for reconstituted than for formic acid reconstituted corn. A similar significant relationship existed between microwaved and steamed corn.

For the most part, increases in DESN occurred with the 24 hr. incubation compared to the 12 hr. incubation (table 4.5). Although differences in DESN due to altering corn grain at different moisture contents were not significant (P>.05), trends in the DESN and IDDM were similar. Significantly (P<.05) lower DESN occurred for reconstituted and microwaved corn compared to formic acid reconstituted and steamed corn, respectively.

Significant (P<.05) increases in ESN and SHSN that occurred with the 24 hr. incubation were consistent with the decrease (P<.05) in residual nitrogen. With the 12 hr. incubation, differences in ESN, SHSN and residual nitrogen due to altering corn grain at different moisture contents were significant (P<.05). Differences in ESN and SHSN between reconstituted and formic acid reconstituted corn and between microwaved and steamed corn were significant (P<.05). Values for residual nitrogen were significantly (P<.05) higher for reconstituted than for formic acid reconstituted corn. Similar significant differences existed for the higher residual nitrogen of microwaved compared to steamed corn.

Discussion

Differences in the ruminal utilization of the carbohydrate and nitrogen fractions of altered corn grain occurred between the 12 and 24 hr. incubations. For the most part, differences among methods of alteration that existed at 12 hr. remained essentially unchanged after 24 hr. incubation. Although the increase in total carbohydrate utilization from the 12 to 24 hr. incubation was lower for ensiled corn than for heat treated corn (1.8 vs. 2.5, respectively), utilization of the total carbohydrates of ensiled corn was essentially complete at the 24 hr. incubation which is the result of higher initial utilization. Utilization of the α-amylase and β-amylase soluble carbohydrates by rumen microorganisms decreased with increased incubation which suggests that either further starch breakdown or microbial synthesis had occurred. Volatile fatty acid production increased from the 12 to the 24 hr. incubation and is not in agreement with data reported by Helm, Lane and Leighton (1972).

Similar increases in total soluble nitrogen existed for ensiled and heat treated (2.6 vs. 2.4, respectively) between the 12 and 24 hr. incubations. The higher total soluble nitrogen for the ensiled corn suggests that a greater proportion of the insoluble matrix protein was hydrolyzed by the rumen microorganisms. Lower ammonia nitrogen levels were associated with increased incubation which indicates that either the rate of hydrolysis is reduced or that the rate of microbial protein synthesis exceeds hydrolysis. With the 24 hr. incubation, values for ethanol and sodium hydroxide soluble nitrogen were higher for ensiled corn compared to heat

treated corn which suggests that increased hydrolysis of the protein matrix occurred.

When comparisons are made with dried corn rather than untreated corn the differences in dry matter digestibility of artificially altered corn grain are of lesser magnitude but agree with those previously reported (Husted et al., 1968; Buchanan-Smith, Totusek and Tillman, 1968; Riggs and McGinty, 1970). With the exception of formic acid reconstituted corn, digestibilities for altered corn were lower than for untreated corn. The data suggest that the reduction in digestibility due to alteration of the grain is related to changes in carbohydrate and nitrogen solubility of the altered grain (Danley and Vetter, 1972b,c).

Total carbohydrate digestibility closely paralleled digestible dry matter. This is expected since carbohydrates comprise approximately 75% of the grain dry matter. The involvement of added moisture in the reconstituted and steamed corn appears to be important in affecting total carbohydrate utilization by rumen microorganisms. McNeill, Potter and Riggs (1971) studied ruminal and postruminal carbohydrate utilization of processed sorghum grain and reported significantly higher ruminal digestion of the total carbohydrates for reconstituted and steam-flaked grains. The data tend to confirm their findings; however, in this study, utilization of the total carbohydrates of steamed corn is of a lesser magnitude. The importance of flaking and the degree of flaking following steaming on the susceptibility of starch to enzymatic attack has been demonstrated by Osman et al. (1970). The steamed corn used in this study was not flaked but its carbohydrates were more susceptible to enzymatic attack than either the carbohydrates of microwaved or reconstituted corn (Danley and

Vetter, 1972b). With processes that involve gelatinization of starch, it

is possible to produce starch derived dextrins which are fairly soluble

but resistant to enzymatic attack (Walker, 1966). This may account for

the lower total carbohydrate utilization of the steamed and microwaved

grains. The data suggest that factors other than susceptibility of the

carbohydrates to enzymatic attack are involved in ruminal utilization of

the total carbohydrates. The lower total carbohydrate utilization of

the reconstituted compared to formic acid reconstituted corn is possibly

the result of decreased substrate availability or of inactivation of the

endogenous enzymes due to drying before reconstitution. Compared with

untreated ensiled corn, higher carbohydrate utilization of reconstituted

and formic acid reconstituted corn may be due to the swelling and sub-

sequent rupture of the starch molecules caused by hydration or to the

greater effect of the endogenous enzyme activity of the reconstituted

and formic acid reconstituted corn on the solubilization of starch. The

data are in agreement with that reported by Karr, Little and Mitchell

(1966) which indicates that considerable quantities of starch may escape

utilization in the rumen which is then available postruminally. Data

reported by Little, Mitchell and Reitnour (1968) suggest that there is a

biological maximum to postruminal carbohydrate or starch utilization. As

a result of lower ruminal carbohydrate utilization of the heat treated

corn compared to ensiled corn, the need for greater postruminal carbo-

hydrate utilization would be exaggerated if total carbohydrate utilization

is to remain unchanged.

Regardless of method of alteration, ruminal utilization of the

α-amylase and β-amylase soluble carbohydrates was high and represented

a source of readily available carbohydrates for either microbial growth or volatile fatty acid production. Interpretation of the volatile fatty acid data is difficult since peaks of production would not occur at the same time for each of the altered grains. For the most part, higher acetate and lower propionate production was associated with increased utilization of total carbohydrates which resulted in narrower acetate:propionate ratios for the heat treated corn. Higher valerate production occurred with the ensiled corn which is in agreement with data reported by Lane, Leighton and Bade (1972).

The data suggest that the method of artificially altering corn also affects ruminal utilization of the grain nitrogen. Differences in total soluble nitrogen and the solubility of the total insoluble nitrogen after incubation indicate that differences in the ruminal utilization of nitrogen occurred among the methods of alteration. Ruminal utilization of grain nitrogen and subsequent synthesis of high quality microbial protein is reduced if nitrogen is not readily available or is insoluble to any great extent (Burroughs et al., 1950). Although total soluble nitrogen contents of artificially altered corn after incubation were low, they were directly related to in vitro digestible dry matter. Data reported by McDonald (1952) indicate that the rate of proteolysis and level of free ammonia in the rumen are proportional to protein solubility of the ration. Ammonia nitrogen levels were lowest for ensiled corn compared to heat treated corn which suggests that the rate of proteolysis and synthesis of microbial protein is affected by the method of alteration.

In this study, the water soluble nitrogen fraction was most affected by the method of alteration (Danley and Vetter, 1972c). Water soluble

nitrogen was directly related to total soluble nitrogen after incubation and to *in vitro* digestible dry matter with higher values for ensiled corn compared to heat treated corn.

Following digestion by rumen microorganisms, dietary protein leaves the rumen either as ammonia, microbial protein or undigested food protein (McDonald, 1954). Similarly, protein entering the abomasum that is not soluble in ethanol would consist of microbial protein and nonzein protein of the undigested food residue. If this assumption is valid, differences in the ethanol soluble nitrogen content before and after incubation with rumen microorganisms would provide an estimate of the ruminal utilization for zein nitrogen. The data indicate that heat treatment of corn grain significantly reduces ruminal utilization of the ethanol soluble nitrogen. Ely *et al*. (1967) suggest that the rate of proteolysis rather than the uptake of released nitrogen limits conversion of zein to microbial protein. However, the higher levels of ammonia associated with heat treated grains suggest that uptake of released nitrogen is also affected.

Heat treated grain is thought to have a higher metabolizable protein value because of its lower nitrogen solubility (Burroughs, Trenkle and Vetter, 1971). The lower soluble nitrogen contents of the heat treated corn after incubation, particularly the microwaved corn, indicate that ruminal utilization of the heat treated corn nitrogen was reduced. Potter, McNeill and Riggs (1971) reported lower ruminal protein utiliza- tion of dried and microwaved sorghum compared to reconstituted and steam flaked grain which would result in more of the grain protein escaping ruminal degradation. However, the possibility that rumen fermentation is increased resulting in more microbial protein of higher biological

value being available for postruminal absorption with ensiled grains should not be overlooked.

The data suggest that the method of artificially altering corn grain has similar effects on the utilization of the carbohydrates and nitrogen of altered grain by rumen microorganisms. Ruminal utilization of the carbohydrates and nitrogen appears to be increased with ensiled corn and reduced with heat treated corn. Ørskov, Fraser and McDonald (1971) reported similar data which suggests that a decrease in the extent of utilization of grain carbohydrates is associated with a decrease in ruminal utilization of grain nitrogen.

The data reported herein further indicate that the method of artificially altering corn grain affects ruminal utilization of the carbohydrate and nitrogen fractions of the grain.

Summary

Changes in the *in vitro* utilization of the carbohydrate and nitrogen fractions of artificially altered corn grain were determined. Field corn (Pioneer 3570), harvested at 22, 18 and 16% moisture, was altered by the following methods: untreated-ensiled, dried reconstituted-ensiled, formic acid reconstituted-ensiled, dried, microwaved and steamed.

For the most part, significant (P<.05) differences occurred between 12 and 24 hr. incubations of corn grain harvested at 22% moisture and artificially altered; however, differences among methods of alteration that existed at 12 hr. remained unchanged.

With the 12 hr. incubation, ensiled corn had significantly (P<.05) higher *in vitro* digestible dry matter (IDDM) and digestible total

carbohydrates (DTCHO). Significant (P<.05) differences existed between reconstituted and formic acid reconstituted corn and between microwaved and steamed corn. Differences in digestible α-amylase and β-amylase soluble carbohydrates were not significant (P>.05). Acetate production for heat treated corn was significantly (P<.05) lower and propionate production significantly (P<.05) higher than for ensiled corn. Differences in volatile fatty acid production between reconstituted and formic acid reconstituted corn and between microwaved and steamed corn were significant (P<.05).

With the 12 hr. incubation, total soluble nitrogen was significantly (P<.05) lower and ammonia nitrogen and total insoluble nitrogen were significantly (P<.05) higher for reconstituted compared with formic acid reconstituted corn and for microwaved compared with steamed corn. Significant (P<.05) differences in TSN, ethanol and sodium hydroxide soluble nitrogen and residual nitrogen occurred due to altering corn grain at different moisture contents. Values for residual nitrogen were significantly (P<.05) higher for reconstituted and microwaved corn compared with reconstituted and steamed corn, respectively.

TABLE 4.1. IN VITRO UTILIZATION OF THE DRY MATTER AND TOTAL CARBOHYDRATE FRACTIONS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Original moisture level, %	Incu-bation time (hr.)	Unaltered	Method of alteration					
			Ensiled			Heat treated		
			Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
In vitro digestible dry matter, $\%$[b,c,d,e]								
22	12	53.4	47.8	48.9	55.1	41.8	37.5	41.9
22	24	76.6	73.2	76.1	79.5	64.6	55.4	66.0
22	48	91.5	88.6	90.6	93.3	87.7	83.9	88.1
18	12	51.8	49.5	51.1	52.2	42.5	39.7	42.8
16	12	49.3	46.7	47.4	56.5	43.4	41.3	45.0
In vitro digestible total carbohydrates, $\%$[b,c,d,e]								
22	12	52.5	46.2	50.6	55.1	34.6	18.0	35.8
22	24	90.8	90.1	90.7	90.7	73.5	70.6	77.0
18	12	51.3	47.8	49.7	53.4	38.2	27.6	40.4
16	12	50.2	47.1	48.1	58.8	40.6	31.6	43.5

[a]Values for digestible dry matter expressed on a dry matter basis. Values for total soluble carbohydrates expressed as percent of initial carbohydrate.

[b]Values for the longer incubation periods significantly (P<.05) different than the 12 hr. incubation.

[c]Values for artificially altered corn significantly (P<.05) different than those for untreated corn.

[d]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[e]Values for reconstituted corn significantly (P<.05) different than those for formic acid reconstituted corn. Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 4.2. IN VITRO UTILIZATION OF THE α- AND β-AMYLASE SOLUBLE CARBOHYDRATE FRACTIONS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Original moisture level, %	Incubation time (hr.)	Unaltered	Method of alteration					
			Ensiled			Heat treated		
			Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
In vitro digestible α-amylase soluble carbohydrates, %								
22	12	98.5	95.7	95.8	96.0	95.1	93.1	94.5
22	24	89.5	83.5	84.4	79.5	88.2	87.1	87.8
18	12	98.7	96.4	96.5	97.0	96.7	93.5	93.7
16	12	99.1	97.8	97.2	97.8	97.9	93.9	92.9
Residual α-amylase soluble carbohydrates, %[b,c,d,e,f]								
22	12	0.66	0.72	0.66	0.59	1.12	1.72	2.18
22	24	23.55	14.74	13.10	9.60	6.71	8.95	13.45
18	12	0.50	0.65	0.50	0.40	0.81	1.76	2.58
16	12	0.33	0.41	0.40	0.33	0.53	1.77	3.01
In vitro digestible β-amylase soluble carbohydrates, %								
22	12	96.2	94.0	94.6	96.4	96.4	93.7	92.7
22	24	93.2	89.8	91.9	95.1	90.9	94.4	97.8
18	12	95.5	93.9	95.0	96.4	94.9	93.2	81.9
16	12	94.5	93.2	94.3	95.8	95.1	91.5	78.4
Residual β-amylase soluble carbohydrates, %[b,c,d,e,f]								
22	12	1.03	1.28	1.27	0.97	0.91	2.56	4.81
22	24	9.71	11.62	10.14	6.45	4.35	6.39	4.08
18	12	1.22	1.27	1.18	1.11	1.04	2.47	8.78
16	12	1.46	1.59	1.38	1.14	1.10	2.36	9.46

[a]Values for digestible α and β soluble carbohydrates expressed as percent of initial α and β soluble carbohydrate contents on dry matter basis. Values for residual α and β soluble carbohydrates expressed as percent of residual carbohydrate remaining after digestion on dry matter basis.

[b]Values for 24 hr. incubation significantly (P<.05) different than for 12 hr. incubation.

[c]Significant (P<.05) differences due to decreasing moisture content at harvest.

[d]Values for artificially altered corn significantly (P<.05) different than those for untreated corn.

[e]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[f]Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 4.3. IN VITRO VOLATILE FATTY ACID PRODUCTION OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Original moisture level, %	Incu-bation time (hr.)	Unaltered	Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed

Method of alteration — Ensiled: Ensiled, Reconstituted, Formic acid reconstituted — Heat treated: Dried, Microwaved, Steamed

Acetate, mg/g[b,d,e,f]

Original moisture level, %	Incu-bation time (hr.)	Unaltered	Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
22	12	152.1	127.3	131.2	159.8	124.7	113.8	121.0
22	24	283.8	275.2	296.1	333.0	198.0	141.1	220.6
18	12	144.3	139.2	137.8	152.0	125.1	115.1	123.2
16	12	135.5	122.6	142.6	151.7	125.3	117.3	128.8

Propionate, mg/g[b,c,d,e,f]

Original moisture level, %	Incu-bation time (hr.)	Unaltered	Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
22	12	84.2	103.7	97.1	85.6	133.0	152.8	138.3
22	24	138.4	150.2	139.1	122.6	121.9	149.1	120.0
18	12	96.7	109.1	101.2	82.5	126.4	148.6	128.2
16	12	108.0	135.1	106.7	79.6	119.7	139.4	117.2

Acetate:propionate ratio

Original moisture level, %	Incu-bation time (hr.)	Unaltered	Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
22	12	1.81	1.23	1.35	1.87	0.94	0.74	0.88
22	24	2.05	1.83	2.13	2.64	1.62	0.95	1.84
18	12	1.49	1.28	1.36	1.84	0.99	0.77	0.96
16	12	1.25	0.91	1.34	1.90	1.04	0.84	1.10

Butyrate, mg/g[b,c,d,f]

Original moisture level, %	Incu-bation time (hr.)	Unaltered	Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
22	12	72.9	65.0	68.5	78.3	19.3	18.4	44.0
22	24	110.8	110.1	120.9	155.0	100.7	91.3	89.0
18	12	52.4	34.8	51.6	59.7	30.0	25.0	48.7
16	12	38.0	32.1	40.6	44.1	36.1	39.7	57.7

Valerate, mg/g^{b,c,d,f}

22	12	18.5	17.6	19.4	21.2	7.0	5.2	8.8
22	24	23.1	28.0	29.8	33.8	20.7	18.9	21.1
18	12	13.4	19.9	20.5	18.3	7.8	6.1	10.5
16	12	12.2	15.1	17.2	22.6	8.0	7.2	12.2

Isovalerate, mg/g^{b,d,f}

22	12	14.9	11.9	13.4	15.9	7.7	7.2	7.8
22	24	18.2	17.6	19.5	23.4	15.9	13.1	15.4
18	12	13.1	12.0	14.2	14.8	7.8	7.3	7.8
16	12	12.5	10.2	12.2	16.0	7.8	7.3	7.9

[a]Values expressed on a dry matter basis.

[b]Values for 24 hr. incubation significantly (P<.05) different than for 12 hr. incubation.

[c]Significant (P<.05) differences due to decreasing moisture content at harvest.

[d]Values for artificially altered corn significantly (P<.05) different than those for untreated corn.

[e]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[f]Values for reconstituted and formic acid reconstituted corn significantly (P<.05) different than those for untreated-ensiled corn. Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 4.4. IN VITRO UTILIZATION OF THE NITROGEN FRACTIONS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

Original moisture level, %	Incu-bation time (hr.)	Unaltered	Method of alteration					
			Ensiled			Heat treated		
			Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
				In vitro total soluble nitrogen, $\%^{b,c,d}$				
22	12	10.7	8.8	10.8	11.9	7.4	5.5	8.3
22	24	26.0	22.8	25.2	32.8	17.5	12.2	20.2
18	12	9.6	9.1	10.1	11.3	8.2	7.0	8.8
16	12	8.1	7.1	8.1	14.3	8.4	8.0	9.2
				In vitro ammonia nitrogen, $\%^{d}$				
22	12	53.2	53.1	56.6	43.5	60.0	70.1	55.3
22	24	52.2	60.3	56.0	42.8	57.4	68.8	54.6
18	12	52.0	46.9	57.7	45.6	59.8	65.4	54.8
16	12	50.8	59.2	60.3	46.5	57.8	60.9	53.0
				In vitro total insoluble nitrogen, $\%^{b,d}$				
22	12	84.1	90.9	86.9	80.3	89.1	94.4	89.0
22	24	71.9	74.0	70.0	65.6	77.7	84.4	76.8
18	12	86.5	89.5	86.0	83.6	87.3	90.0	88.2
16	12	88.7	91.4	88.8	85.0	89.8	90.2	87.7

[a]Total soluble nitrogen values expressed as percent of initial total nitrogen. Ammonia nitrogen values expressed as percent of soluble nitrogen. Total insoluble nitrogen expressed as percent of initial total nitrogen.

[b]Values for 24 hr. incubation significantly (P<.05) different than for 12 hr. incubation.

[c]Significant (P<.05) differences due to decreasing moisture content at harvest.

[d]Values for reconstituted corn significantly (P<.05) different than those for formic acid reconstituted corn. Values for microwaved corn significantly (P<.05) different than those for steamed corn.

TABLE 4.5. IN VITRO UTILIZATION OF THE ETHANOL AND SODIUM HYDROXIDE SOLUBLE AND RESIDUAL NITROGEN FRACTIONS OF ARTIFICIALLY ALTERED CORN GRAIN HARVESTED AT THREE MOISTURE LEVELS[a]

| Original moisture level, % | Incu-bation time (hr.) | Unaltered | Method of alteration | | | | | |
| | | | Ensiled | | | Heat treated | | |
			Ensiled	Reconstituted	Formic acid reconstituted	Dried	Microwaved	Steamed
			In vitro digestible ethanol soluble nitrogen, %[b,d,e]					
22	12	56.3	42.8	49.8	64.9	24.0	22.0	33.9
22	24	50.7	46.2	54.8	60.8	34.2	24.1	37.4
18	12	52.8	38.7	46.4	63.6	23.4	17.7	26.9
16	12	53.4	42.1	49.4	69.6	16.1	9.8	22.3
			Residual ethanol soluble nitrogen, %[b,c,d,e]					
22	12	10.9	14.8	14.2	11.2	15.6	9.0	15.3
22	24	14.4	17.1	15.9	15.3	15.5	9.8	16.8
18	12	12.0	15.2	14.4	10.7	17.8	11.8	18.3
16	12	12.2	14.7	13.4	8.7	19.8	15.7	20.2
			Residual sodium hydroxide soluble nitrogen, %[b,c,d,e]					
22	12	43.2	38.1	40.5	54.0	37.4	27.3	38.3
22	24	63.1	58.2	67.0	76.8	49.4	40.6	53.6
18	12	46.0	41.4	46.6	54.2	40.4	30.8	41.1
16	12	48.4	44.5	49.8	53.8	41.3	32.8	44.0
			Residual nitrogen, %[b,c,d,e]					
22	12	45.4	46.6	45.2	33.8	47.0	63.7	46.4
22	24	22.5	24.7	17.1	7.9	35.1	49.7	29.6
18	12	42.0	47.0	39.0	35.1	41.8	57.4	40.5
16	12	39.4	40.8	36.8	37.5	38.9	51.4	35.8

[a]Values for digestible ethanol soluble nitrogen expressed as percent of initial ethanol soluble nitrogen on dry matter basis. Residual ethanol and sodium hydroxide soluble nitrogen and residual nitrogen values expressed as percent of total insoluble nitrogen.

[b]Values for 24 hr. incubation significantly (P<.05) different than for 12 hr. incubation.

[c]Significant (P<.05) differences due to decreasing moisture content at harvest.

[d]Values for ensiled corn significantly (P<.05) different than those for heat treated corn.

[e]Values for reconstituted corn significantly (P<.05) different than those for formic acid reconstituted corn. Values for microwaved corn significantly (P<.05) different than those for steamed corn.

SECTION V:

EFFECTS OF STORAGE TIME AND FORMIC ACID ON

THE CHEMICAL COMPOSITION AND DIGESTIBILITY

OF RECONSTITUTED HIGH MOISTURE CORN

Introduction

The most important factors related to the effective preservation of a feedstuff by ensiling are the maintenance of a relatively anaerobic atmosphere, an ideal moisture level and the development of an acid condition. The development of acidity seems to be of less significance if the requirements of anaerobiosis and moisture level are met. Generally, anaerobiosis and moisture level are not optimum so there has been much effort to provide a more effective acidity by the use of additives to regulate microbial activity and to minimize the production of undesirable end products of fermentation.

The processes that occur in grain during reconstitution are thought to resemble those that occur during germination and have been reviewed by Riggs (1969). Data reported by Danley and Vetter (1972b,c,d) indicate that formic acid significantly affects the fermentation and utilization of corn grain. Hale et al. (1969) and Wagner (1970) reported that optimum responses were obtained when sorghum grain was reconstituted to 30% moisture and stored for a minimum of 20 days in an airtight structure. In contrast, Pantin, Riggs and Bowers (1969) reported that 10 days storage was adequate. Similar data with corn grain are not readily available.

In view of the absence of available information on the effects of reconstitution of corn grain, this research was conducted to determine the effects of length of storage and formic acid on the changes in composition and utilization of reconstituted corn grain.

Materials and Methods

Nine 3 kg samples of field dried (12% moisture) shelled corn were reconstituted to 35% moisture with distilled water at 4 C. The reconstituted samples were randomly assigned to the following storage periods: (1) 5 days, (2) 10 days and (3) 15 days. The effect of storage time was studied using the laboratory silo apparatus described by Otis, Pomroy and Gawreluk (1959) as modified by Danley and Vetter (1972b). The three storage periods were randomly assigned within each group of three airtight, plexiglass silos. A constant pressure of 527 g/cm^2 and a constant temperature of 32 C were maintained during storage. Volatile gasses produced during storage were absorbed in 1 N KOH and 4% H_3BO_3.

A second group of nine 3 kg samples of field dried (12% moisture) shelled corn was reconstituted to 33% moisture with distilled water at 4 C. Formic acid (88% solution) was added at 0.5% of the fresh weight following reconstitution. The formic acid reconstituted samples were randomly assigned to the 5, 10 and 15 day storage periods and stored as previously described for the reconstituted corn samples.

Fresh and stored high moisture corn grain samples were freeze-ground as described by Danley and Vetter (1971). Duplicate samples were chemically analyzed in the freeze-ground state for dry matter (DM), total carbohydrate (TCHO), water soluble carbohydrates (SCHO), α-amylase soluble carbohydrates (α-SCHO), β-amylase soluble carbohydrates (β-SCHO) and insoluble carbohydrates (ISCHO) as described by Danley and Vetter (1972a). Total nitrogen (TN), water soluble nitrogen (WSN), sodium chloride soluble nitrogen (SCSN), ethanol soluble nitrogen (ESN), sodium hydroxide soluble nitrogen (SHSN),

ammonia nitrogen (NH_3-N) and α-amino nitrogen (NH_2-N) were determined as
outlined by Danley and Vetter (1972b). Digestible dry matter (IDDM) was
determined after 24 hr. by the two-stage _in vitro_ digestion procedure of
Tilley and Terry (1963).

The data were analyzed statistically by least-squares analysis of
variance for a completely randomized design, followed by orthogonal com-
parisons of treatment means as outlined by Snedecor and Cochran (1967).

Results and Discussion

Dry matter (DM), total nitrogen (TN) and gaseous losses from the
storage of reconstituted corn grain are presented in table 5.1. Differ-
ences in DM, TN and gaseous losses for 5 _vs_. 10 and 15 days storage were
significant ($P<.05$). Differences between 10 and 15 days storage approached
significance ($P<.05$) which indicates that larger losses occurred during the
early stages of storage either by plant respiration or by microbial activ-
ity. The data indicate that increased losses are associated with increased
storage time. Similar responses have been reported by Dexter (1966) and
Barnett (1954). The increase in moisture content of reconstituted corn
grain with length of storage indicates that water is liberated during
storage. Data reported by Nagel and Semeniuk (1947) suggest that the
increase in moisture may be attributed to the metabolic activities of the
microorganisms present.

Formic acid reconstituted corn grain had significantly ($P<.05$) lower
DM, TN and gaseous losses than reconstituted corn. Lower dry matter losses
of formic acid treated grass silage have been reported by Derbyshire,
Waldo and Gordon (1971) and Danley and Vetter (1972b). The lower initial

CO_2 and volatile nitrogen losses of the formic acid reconstituted corn suggest that the lower initial pH (Danley, unpublished data) reduced the multiplication of proteolytic and butyric acid producing organisms and minimized the initial oxidative losses.

Length of storage and formic acid had no significant ($P>.05$) effect on the TCHO content (table 5.2). Differences in SCHO for 5 vs. 10 and 15 days storage and between 10 and 15 days storage were significant ($P<.05$); however, differences in ISCHO were only significant ($P<.05$) between 10 and 15 days. Regardless of the length of storage, formic acid reconstituted corn had a significantly ($P<.05$) higher SCHO content.

The difference in the initial decline of SCHO between reconstituted and formic acid reconstituted shelled corn indicates that lower initial oxidative losses occurred with the formic acid reconstituted corn and is consistent with the differences in CO_2 loss. The increase in SCHO with increasing length of storage is not in agreement with data reported for forage crops (Barnett, 1954; McDonald, Henderson and MacGregor, 1968) and that previously reported for reconstituted corn grain (Danley and Vetter, 1972b). Compared with forages, the increased SCHO content of reconstituted grain is the result of an increased supply of carbohydrates that are available for microbial breakdown. Ingle, Beevers and Hageman (1964) reported increased α-amylase activity during the latter stages of germination. Their data indicate that the rate of hydrolytic breakdown of endosperm starch exceeded the rate of utilization by the developing seedling and resulted in an increase of soluble carbohydrate with time. Similar increased amylase activity during the storage of dry grains has been reported (Zeleny, 1948). The decreased reactivity of dried corn

starch reported by Whistler, Goatley and Spencer (1959) could account for the lower SCHO content previously reported for reconstituted corn grain (Danley and Vetter, 1972b).

The effect of length of storage on the α-SCHO, β-SCHO and IDDM is shown in table 5.3. Regardless of storage time, formic acid reconstituted corn grain had significantly (P<.05) lower α-SCHO content than did reconstituted corn. This effect may be attributed to the inactivation of the α-amylase enzyme at the lower pH of the formic acid treated corn. A significant (P<.05) reduction in α-SCHO occurred initially with reconstituted corn grain, but there was no significant (P>.05) effect due to length of storage. The initial reduction of α-SCHO indicates that this fraction serves as a readily utilizable energy source for the developing microflora. Length of storage or formic acid had no significant (P>.05) effect on β-SCHO. Similar results reported by Hale (1971) indicate that gelatinization of the starch granule does not occur with reconstitution.

Formic acid had no significant (P>.05) effect on IDDM (table 5.3). The increase in IDDM due to storage was significant (P<.05), but the differences in IDDM between 10 and 15 days storage were not significant (P>.05). Similar results for reconstituted milo have been reported by Pantin, Riggs and Bowers (1969).

Formic acid had no significant (P>.05) effect on either the WSN, SCSN or ESN contents of reconstituted corn grain (table 5.4). Increases in WSN, SCSN and ESN with increased storage time and differences for 5 vs. 10 and 15 days storage were significant (P<.05). Differences between 10 and 15 days storage, however, were not significant (P>.05).

The effect of length of storage on the SHSN, TSN, ISN, NH_3-N and NH_2-N is shown in table 5.5. The increase in SHSN and TSN and the decrease in ISN contents with increased storage time were significant (P<.05). Although differences in TSN and ISN for 5 vs. 10 and 15 days storage were significant (P<.05), no significant (P>.05) differences existed between 10 and 15 days storage. Formic acid had no significant (P>.05) effect on either SHSN, TSN or ISN.

The largest percentage change in nitrogen solubility due to storage of reconstituted corn grain occurred with WSN fraction. Similar increases in soluble nitrogen of germinating corn have been reported (Ingle, Beevers and Hageman, 1964). Duvick (1961) suggests that the increased soluble nitrogen originates from the hydrolysis of zein found in the protein storage bodies of the endosperm. Data reported by Hale (1971) indicates that disruption of the protein matrix occurs during reconstitution which could account for increased solubility of total nitrogen.

Formic acid reconstituted corn grain had significantly (P<.05) lower NH_3-N than reconstituted corn (table 5.5). Differences in NH_3-N for 5 vs. 10 and 15 days storage were significant (P<.05). Compared to reconstituted corn, the decrease in NH_3-N of formic acid reconstituted corn due to storage indicates that less hydrolysis of the soluble nitrogen occurred. Similar lower NH_3-N contents of formic acid treated grass silages have been reported by Waldo et al. (1969). Differences in NH_2-N due to storage and for 5 vs. 10 and 15 days storage were significant (P<.05) which suggests that considerable breakdown of the insoluble nitrogen occurs with reconstitution. Increases in NH_2-N with increased storage time indicates that hydrolysis of the insoluble endosperm protein is a continual process.

Differences in the NH_2-N content between reconstituted and formic acid reconstituted corn approached significance (P<.05). The higher NH_2-N content of the reconstituted corn is associated with higher NH_3-N levels and possibly serves as a source of NH_3-N.

The data reported herein are in agreement with data reported by Riggs (1969) in that the processes that occur during reconstitution closely resemble those occurring during germination. Associated with the uptake of water by the whole grain there was an increase in both soluble carbohydrates and soluble nitrogen. Consistent with the processes occurring during germination, solubilization of nitrogen began during the initial phase of reconstitution with essentially no change in total nitrogen. The decrease in soluble carbohydrates in the initial phase of reconstitution followed by an increase with increasing length of storage indicates that hydrolysis of the reserve carbohydrates is not initiated during the early phase of reconstitution.

The increased solubilization of insoluble endosperm carbohydrates and nitrogen that occurs during germination is thought to be due to hormone induced production of endogenous enzymes (Van Overbeek, 1966). However, the environmental conditions that exist during reconstitution approach those that are optimum for the development of an active microflora (Burmeister, Hartman and Saul, 1966; Burmeister and Hartman, 1966). The effect of a mixed microflora on the solubilization of carbohydrates and nitrogen of corn grain have been reported by Nagel and Semeniuk (1947). It appears that solubilization of the insoluble carbohydrates and nitrogen of the endosperm during reconstitution is the result of the combination of

endogenous and microbial enzyme activity and that at least 10 days of storage are necessary for the completion of this solubilization.

Summary

Research was conducted to determine the effects of storage time (5, 10 and 15 days) and formic acid on the chemical composition and digestibility of reconstituted corn grain.

Although dry matter (DM), total nitrogen (TN) and gaseous losses increased with storage time, differences between 10 and 15 days storage were not significant $(P>.05)$. Formic acid reconstituted corn had significantly $(P<.05)$ lower DM, TN and gaseous losses than reconstituted corn.

The total carbohydrate content of reconstituted corn was not significantly $(P>.05)$ affected by storage time or formic acid, but the increase in soluble carbohydrates was significant $(P<.05)$. A significant $(P<.05)$ reduction in α-amylase soluble carbohydrates occurred initially during storage and with the formic acid treatment. Neither length of storage nor formic acid had a significant $(P<.05)$ effect on the susceptibility of starch to β-amylase. _In vitro_ digestible dry matter increased with storage time but no significant $(P>.05)$ difference between 10 and 15 days storage was observed.

Although water, sodium chloride, ethanol, sodium hydroxide and total soluble nitrogen increased significantly $(P<.05)$ with the storage of both reconstituted and formic acid reconstituted corn, differences between 10 and 15 days storage were not significant $(P>.05)$. Formic acid reconstituted corn had significantly $(P<.05)$ lower ammonia nitrogen (NH_3-N) than

reconstituted corn. Differences in NH_3-N and α-amino nitrogen due to storage were significant ($P<.05$), but no significant ($P>.05$) differences between 10 and 15 days storage were observed.

TABLE 5.1. EFFECT OF STORAGE TIME AND FORMIC ACID ON THE DRY
MATTER (DM), TOTAL NITROGEN (TN) AND GASEOUS LOSSES
OF RECONSTITUTED SHELLED CORN[a]

Item	Reconstituted			Formic acid reconstituted		
	Days of storage					
	5	10	15	5	10	15
Initial weight, g	2790	2771	2755	2799	2878	2821
Final weight, g	2718	2699	2670	2793	2850	2794
Loss, %	2.58	2.60	3.08	0.31	0.97	0.96
Initial DM, %[b]	65.4	66.0	65.9	67.0	68.8	68.9
Final DM, %[b]	64.8	64.9	64.8	67.1	68.4	68.4
DM loss, %[b,e]	3.42[c]	4.26[c]	5.38[c]	1.27[d]	1.58[d]	1.76[d]
Initial TN, mg/g[b]	14.1	14.3	14.3	13.4	13.5	13.6
Final TN, mg/g[b]	14.4	14.8	14.8	13.5	13.6	13.7
TN loss, %[b,e]	1.08[c]	1.46[c]	1.99[c]	0.46[d]	1.11[d]	1.47[d]
CO_2 loss, mg/g[b,e]	5.4[c]	6.8[c]	7.2[c]	3.2[d]	3.8[d]	4.2[d]
Volatile N, µg/g[b,e]	152[c]	209[c]	394[c]	62[d]	150[d]	201[d]

[a]Values expressed as averages of the three silos per treatment.

[b]Values expressed on dry matter basis.

[c,d]Values with different superscripts on the same line differ
significantly (P<.05).

[e]Significant (P<.05) difference for 5 vs. 10 and 15 days storage.

TABLE 5.2. EFFECT OF STORAGE TIME AND FORMIC ACID (FA) ON THE
TOTAL, WATER SOLUBLE AND INSOLUBLE CARBOHYDRATE
CONTENTS OF RECONSTITUTED SHELLED CORN[a]

Treatment	Days of storage			
	0	5	10	15
Total carbohydrate, mg/g				
Reconstituted	744.1	736.0	740.1	741.0
FA-reconstituted	745.5	752.7	756.9	757.8
Water soluble carbohydrate, $\%$[b,c,d]				
Reconstituted	4.8	3.2	3.7	5.0
FA-reconstituted	5.0	4.9	5.3	6.2
Insoluble carbohydrate, mg/g[d]				
Reconstituted	707.5	712.3	710.6	702.7
FA-reconstituted	707.9	714.8	714.9	710.0

[a]Values expressed on dry matter basis. Water soluble carbohydrates expressed as percent of the total carbohydrate content.

[b]Values for formic acid reconstituted significantly (P<.05) lower than values for reconstituted corn.

[c]Significant (P<.05) differences for 5 vs. 10 and 15 days storage.

[d]Significant (P<.05) differences between 10 and 15 days storage.

TABLE 5.3. EFFECT OF STORAGE TIME AND FORMIC ACID (FA) ON THE
α-AMYLASE AND β-AMYLASE SOLUBLE CARBOHYDRATE CONTENTS
AND IN VITRO DIGESTIBLE DRY MATTER OF RECONSTITUTED SHELLED CORN[a]

Treatment	Days of storage			
	0	5	10	15
α-amylase soluble carbohydrates, $\%^{b,c}$				
Reconstituted	21.1	8.6	8.8	9.0
FA-reconstituted	6.0	4.9	4.7	4.4
β-amylase soluble carbohydrates, $\%$				
Reconstituted	13.8	12.3	12.6	12.8
FA-reconstituted	12.7	12.2	12.4	12.5
In vitro digestible dry matter, $\%^{c,d}$				
Reconstituted	62.5	64.3	72.8	76.5
FA-reconstituted	64.2	66.7	76.2	79.5

[a]Values for α-amylase and β-amylase soluble carbohydrates expressed
as percent of total carbohydrate content on dry matter basis. Digestible
dry matter expressed as percent of the dry matter.

[b]Values for formic acid reconstituted significantly (P<.05) lower
than values for reconstituted corn.

[c]Significant (P<.05) difference due to storage.

[d]Significant (P<.05) difference for 5 vs. 10 and 15 days storage.

TABLE 5.4. EFFECT OF STORAGE TIME AND FORMIC ACID (FA) ON THE WATER, SODIUM CHLORIDE AND ETHANOL SOLUBLE NITROGEN CONTENTS OF RECONSTITUTED SHELLED CORN[a]

Treatment	Days of storage			
	0	5	10	15
Water soluble nitrogen, $\%$[b],[c]				
Reconstituted	11.1	15.2	17.8	20.8
FA-reconstituted	10.6	16.6	19.5	21.6
Sodium chloride soluble nitrogen, $\%$[b],[c]				
Reconstituted	11.1	13.0	13.7	14.8
FA-reconstituted	10.9	12.8	15.0	15.5
Ethanol soluble nitrogen, $\%$[b],[c]				
Reconstituted	18.4	20.6	20.7	21.1
FA-reconstituted	20.9	21.3	22.2	23.5

[a]Values expressed as percent of total nitrogen content on dry matter basis.

[b]Significant ($P<.05$) difference due to storage.

[c]Significant ($P<.05$) difference for 5 vs. 10 and 15 days storage.

TABLE 5.5. EFFECT OF STORAGE TIME AND FORMIC ACID (FA) ON THE
SODIUM HYDROXIDE, TOTAL SOLUBLE, INSOLUBLE, AMMONIA AND
α-AMINO NITROGEN CONTENTS OF RECONSTITUTED SHELLED CORN[a]

Treatment	Days of storage			
	0	5	10	15
Sodium hydroxide soluble nitrogen, $\%^c$				
Reconstituted	23.5	26.2	26.9	27.6
FA-reconstituted	24.4	24.8	27.6	28.8
Total soluble nitrogen, $\%^{c,d}$				
Reconstituted	64.2	74.9	79.1	84.5
FA-reconstituted	66.8	75.5	84.3	89 4
Insoluble nitrogen, $\%^{c,d}$				
Reconstituted	35.8	25.1	20.9	15.5
FA-reconstituted	33.2	24.5	15.7	10.6
Ammonia nitrogen, $\%^{b,c,d}$				
Reconstituted	66.7	68.5	71.9	73.4
FA-reconstituted	65.5	71.6	60.6	51.2
α-amino nitrogen, $mm^{c,d}$				
Reconstituted	5.6	72.5	83.9	95.1
FA-reconstituted	2.5	72.8	78.0	88.6

[a]Values expressed as percent of total nitrogen content on dry matter
basis. Ammonia nitrogen expressed as percent of water soluble nitrogen.
α-amino nitrogen expressed on dry matter basis.

[b]Values for formic acid reconstituted significantly (P<.05) different
than those for reconstituted corn.

[c]Significant (P<.05) difference due to storage.

[d]Significant (P<.05) differences for 5 vs. 10 and 15 days storage.

GENERAL DISCUSSION AND SUMMARY

This research was conducted to determine the changes in the carbo-
hydrate and nitrogen fractions and _in vitro_ utilization of artificially
altered corn grain, and was reported in five sections consisting of data
obtained from seven experiments. In all experiments, corn grain was
artificially altered either by ensiling or by heat treating.

Alteration of the grain by ensiling was achieved by using a modifi-
cation of the Minnesota laboratory silo unit. Although the heating and
pressure systems were modified, variations in temperature and pressure
were similar to those of the Minnesota unit and indicate that these
variables can be maintained within narrow limits. The modifications of
the component parts of each silo appeared not to affect the maintenance
of an anaerobic environment within each silo. Absorption of the volatile
losses of fermentation and the circulating gas system offer the advantage
of exposing the upper and lower surfaces of the ensiling material to a
gas of known composition without correcting for the volume of the circulat-
ing gas. Some improvement over the Minnesota unit occurred with the
absorption and quantitation of the volatile losses of fermentation even
though the results were variable and the differences among the silos
within treatments approached significance ($P<.05$).

The data indicate that alteration of corn grain results in opposing
effects on carbohydrate and nitrogen solubility. Compared to drying,
which decreases both carbohydrate and nitrogen solubility, ensiling and
reconstitution does not appreciably increase carbohydrate solubility
but does increase the solubility of nitrogen. On the other hand, the

carbohydrates of microwaved and steamed corn are more susceptible to
enzymatic attack but with microwaved corn large decreases in nitrogen
solubility occur.

In addition to the water soluble carbohydrate fraction, the carbo-
hydrates that are susceptible to β-amylase were most affected by the
method of alteration. For the most part, the results of these studies
are in agreement with those reported by other workers in that ensiling
and reconstitution result in no gelatinization of the corn starch.
In contrast, microwaving and steaming result in considerable starch
gelatinization. Steaming at a low pressure without flaking resulted
in considerable gelatinization of the corn starch which is not in agree-
ment with data reported by other workers. The reasons for this cannot
be inferred from the data.

The water soluble nitrogen fraction was most affected by the method
of alteration. The increase in water soluble nitrogen with ensiling and
reconstitution indicates that considerable hydrolysis of the insoluble
endosperm nitrogen occurs. On the other hand, heat treatment reduces the
water soluble nitrogen.

As expected, total carbohydrate utilization closely paralleled
digestible dry matter since carbohydrates comprise approximately 75%
of the grain dry matter. The data indicate that factors other than
susceptibility of the carbohydrates to enzymatic attack are involved
in the utilization of the total carbohydrates by rumen microorganisms.
As a result of lower ruminal carbohydrate utilization of the heat
treated corn compared to ensiled corn, the need for greater postruminal

carbohydrate utilization would be exaggerated if total carbohydrate utilization is to remain unchanged.

Ruminal utilization of the ethanol soluble nitrogen was significantly ($P<.05$) reduced by heat treatment of corn grain which would result in more of the grain protein escaping microbial attack by the rumen microorganisms. Thus, heat treated grain is thought to have a higher metabolizable protein value. However, the possibility that rumen fermentation is increased resulting in more microbial protein of higher biological value being available for postruminal absorption with ensiled grains should not be overlooked.

The beneficial effects of formic acid appeared to be in the initial reduction of pH which reduced the oxidative losses occurring during the early stages of the fermentation process. The possibility does exist that a more active microbial population developed with the formic acid treatment during storage which resulted in greater solubilization of the insoluble endosperm carbohydrates and protein. Compared to formic acid reconstituted corn, the lower solubilization and utilization of the endosperm carbohydrates and protein of reconstituted corn is possibly due to a decreased availability of substrate or to the inactivation of endogenous enzymes due to drying before reconstitution.

The results of these studies indicate that the processes that occur during reconstitution closely resemble those occurring during germination. Consistent with the processes of germination is the solubilization of nitrogen during the initial phase of reconstitution with essentially no change in total nitrogen followed by hydrolysis of the reserve carbohydrates during the latter phase of reconstitution. The environmental conditions

that exist during reconstitution approach those that are optimum for the development of an active microflora. It appears that the solubilization of the insoluble carbohydrate and nitrogen of the endosperm during reconstitution is possibly the result of the combination of endogenous and microbial enzyme activity; however, more work is needed to determine the extent of the involvement of each process.

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