# SOME PROBLEMS CONCERNING THE FERMENTATION OF SILAGE

By

Harlow Roesler Bierman



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Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Harlow Roesler Bierman for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

C. H. Eccles
Chairman

W. H. Peck

L. D. Palmer

Date Dec 19 1922

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Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Harlow Roesler Bierman final oral examination for the degree of

Master of Science.

We recommend that the degree of

Master of Science

be conferred upon the candidate.

Chairman

C. H. Eccles

W. G. Holmes

L. D. Palmer

Date Dec 19 1922

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# INTRODUCTION

Silage is a complex and variable product. This complexity and variableness is due to differences in the fermenting materials, the causative agents and the conditions under which fermentation takes place. Silage, as defined by Webster, is the fodder preserved in a silo. He defines a silo as a pit or vat for packing away green fodder so as to exclude air and outside moisture.

Investigational work on silage had its beginning in observations made in practice. In Europe, Johnston (1843), Reihlen (1869), and Goffart (1877) published the results of their observations on the storing of green crops as silage. In the United States, Francis Morris (1876) of Maryland reported similar experimental work. Scientific work on silage was started in Europe by Moser (1870) and Weiske (1873) and in the United States by Fry (1885) and Burrill (1889). Numerous investigators from this time until 1902 experimented with silage with the view of determining the causative agents in normal silage fermentation and the total losses of organic matter in the silo. During the period of 1902-12, the work of Annett and Russell, in 1908, on the causative factors of normal silage fermentation was the only outstanding investigation. From then until the present time numerous silage investigations have been made on the causative factors and chemical changes in normal fermentation.

The problem of the causative factors and chemical changes in abnormal fermentation of silage has not been the subject of extensive experimental work. The aim of this thesis is to answer, in part, some of the questions involved as to the factors concerned in the preservation and abnormal fermentation of silage, and the chemical changes involved. The following questions will be considered:

1. What part does acid have in the preservation of silage?
2. What is the relation between moisture content of silage and the growth of mold?

3. What are the factors necessary for abnormal fermentation of silage?
4. What happens chemically and physically when normal silage undergoes abnormal fermentation?

## REVIEW OF LITERATURE

### History and development of the silo

The silo originally was a pit or room used for the storage of grain. In very early times in Egypt, Italy, Spain and Mexico seed was stored in such rooms. The preservation of green feed in silos was a common practice over 135 years ago in Italy and Greece. Symonds (1786) wrote of Italians preserving fresh leaves for cattle in casks and pits in the ground. Green feed was preserved in pits in Northern Europe where the uncertainty of the weather and the low temperature make it difficult to cure hay. Johnston (1843) published an article on preserving green clover, grass and vetch in pits. Dirt to a depth of a foot or two was thrown on the cover to exclude air. In England, between 1860-70, Samuel Jonas stored green rye and fed the fermented material on an extensive scale The practice of storing green maize in silos was probably begun in 1869 by Adolf Reihlen, a sugar manufacturer of Stuttgart, Germany. He stored corn of the large yellow variety in a silo, the seed of which he had gotten from the United States. He also preserved green beet leaves and beet pulp in silos with success. The results of his work were published in both German and French, which led many farmers in France to build silos. In 1877, Goffart wrote possibly the first book on ensilage which was translated into English and published a few years later in New York. This book gave the results of 25 years experience in preserving green feed. The first silage made in the United States was put up by Manly Miles of Michigan, who built two silos in 1875. From this beginning the use of the silo

has spread rapidly in the United States. In 1882, statements were published by 91 persons who had silos, but undoubtedly there were more silos built by that time. At the present time the silo is found on many thousands of farms in the United States.

## The present status of the silo

The silo has passed the experimental stage and the economy and practicability of this method of preserving fodder has been fully demonstrated. The silo is considered a well established fixture in American farm economy where stock raising is practiced.

## The present status of silage

Silage has become extremely popular as a feed for cattle. It increases the palatability and succulence of the ration. It provides the only means of conserving the whole corn plant and converting it all into a consumable product for livestock. The use of silage has increased with the increase in farm livestock production, especially dairy cattle. Its extensive use has come about entirely upon its own merits.

## The causative factors of normal silage fermentation.

It is a well known fact that when green plants are cut up and placed in a heap, certain physical, chemical and biological changes occur. Esten and Mason pointed out that there is a thin film of juice surrounding each piece of silage, which affords an ideal media for the growth of sugar fermenting yeasts and bacteria. Russell states that when green fodder is put into the silo the cells are alive and their vital functions continue. Plant physiologists consider the cutting of tissue as a factor in activating respiration. These operating factors in normal silage fermentation involve a rapid evolution of heat followed by a

considerable amount of gas, a loss of some dry matter, a change in color, production of aromatic odors, and the formation of organic acids. A thorough understanding of the causative factors in these changes in silage is desired and if farmers understood what happened in the fermentation process, there would be much less spoilage and waste of silage.

The question of what causes the fermentation of silage has been in controversy ever since the first attempt was made to determine the causative factors. One group of investigators have emphasized the importance of respiration of tissue cells as the main factor in normal silage fermentation. A second group of investigators have maintained that micro-organisms are the essential factors. A third group take an intermediate stand, recognizing both of the two former views, but consider that micro-organisms are the most important factor.

Causative factors of heat production

Fry (1885), Woolny, Babcock and H. L. Russell (1900), and E. J. Russell (1908) consider respiration of the plant cells as the important factor in heat production.

Burrill (1889), Griffeths (1894), Esten and Mason (1912), O. W. Hunter (1917), A. R. Lamb (1917), and E. B. Fred, et al (1921) considered micro-organisms to be the important factors in heat production.

Causative factors of acid production

Babcock and H. L. Russell (1900), E. J. Russell, Hart & Willaman (1912), and Samarani (1913) concluded that respiration of plant cells is a factor in acid production.

Woolny, Samarani (1913), Esten and Mason (1912), O. W. Hunter and Bushnell (1916), A. R. Lamb (1917), J. M. Sherman (1916), Hastings (1920), E. B. Fred et al(1921), Heineman and Hixon (1921), C. A. Hunter (1921), and W. C. Frazier (1921) report experimental data which supports the view that micro-organisms as acid producing types of bacteria are the important factors in the production of acid.

Causative factors of alcohol production

Lamb (1917) and Frazier (1921) conclude that alcohol production results from the activity of both plants cells and micro-organisms. Samarani (1913) supports cell respiration and Fred, et al (1921) are inclined to favor micro-organisms as the causative factors in alcohol production.

E. J. Russell (1908) recognized that all three factors, namely, living protoplasm, enzymes and bacteria are involved and he further states that no hypothesis is satisfactory which fails to take into account all three of these factors. Esten and Mason (1912), Sherman and Bechdel (1918), Lamb (1917), and Heineman and Hixon (1921) consider all three factors of more or less importance. Fred, et al (1921) state that accumulating data tend more and more to indicate the dominant influence of bacteria in the production of silage.

A summarization of the above investigations will reveal that micro-organisms, respiration of plant tissues and plant enzyme action all have a part in normal silage fermentation; the importance of each factor, in all probability being in the order named. The fact that the green fodder is teeming with all types of micro-organisms, the protoplasm of the plant is still functioning and plant enzymes are present would suggest that normal silage fermentation is a combination of all three factors. It is inconceivable to imagine that the placing of green fodder into a silo would immediately destroy the live active functioning bodies.

## The losses due to fermentation of silage

The cost of normal silage fermentation is a loss in organic matter. This loss normally varies. The maturity and kind of fodder used are the factors which cause this variation in losses. Any further losses are due to abnormal fermentation, which occur only when there is a deficiency in acid or when air is present. The total losses in dry matter found by the early investigations were larger than those reported by later investigations because the practice at that

time was to put the corn in the silo when less mature and smaller quantities were ensilaged. Also, rectangular silos were used in some of the early investigations which caused the fodder to settle unevenly and allowed the air to permeate the mass. In calculating the losses the abnormal silage was included, in cases, where extremely high losses are reported.

This subject, namely, the losses due to the fermentation of silage, has engaged the attention of the following list of investigators: Moser (1870), Weiske (1873), Weiske and Schulze (1884), Jordan (1884), Armsby and Caldwell (1889), Henry and Woll (1888), Woll (1889), Short (1888), Cooke (1889), Kedsie (1889), Kellner (1889), Woll (1890), New York Experiment Station (1892), King (1894), Babcock and Russell (1900), King (1900), Withycombe (1901), Clements and Russell (1904), Annett and Russell (1908), Russell (1908), Furuglio and Mayer (1913), Eckles (1916), Swanson and Tague (1917), Lamb (1917), Haigh (1918), Dox and Yoder (1920), Hunter (1921), Shaw, Wright and Deysher (1921), Barthel (1921), and Peterson, Fred and Verhulst (1921). They made chemical analyses and demonstrated some of the losses in total dry matter, nitrogen free extract, protein, crude fibre or ash and gain in ether extract.

Losses of dry matter in silage fermentation.

The losses of total dry matter were found to vary from 3 percent to 40 percent in silage fermentation, depending upon the completeness with which air is excluded. However, 40 percent is far from being a typical figure for it indicates extensive abnormal fermentation which is the exception in present methods of ensilage. King (1894) found a loss of only 4.95 percent in total dry matter. Other early investigators found losses of over 20 percent. Kedzie (1889) found a loss of 28 percent in dry matter with green corn ensilaged August 10th while with mature corn ensilaged on September 14th there was only a loss of 3 percent. The present concensus of opinion is that normal silage losses vary from 3 percent to 10 percent depending upon the maturity and kind of fodder used.

Loss of nitrogen free extract in silage fermentation.

It is agreed among the numerous investigators that the heaviest losses in dry matter falls on the nitrogen free extract. This loss involves the destruction of sugar and other convertible carbo-hydrate materials, with the liberation of carbon-dioxide. Dox and Yoder (1920) conclude that there is no loss of starch in normal fermentation. Fred, Peterson and Verhulst (1921), conclude that from 15-20 percent of the loss of dry matter is loss of pentosans and that the loss of nitrogen free extract, as determined by various experiment stations, varies from 10 to 55 percent.

Loss of protein in silage fermentation.

Kellner (1889) demonstrated that the decrease in protein was accompanied by an increase in amide nitrogen. He considered that there was no loss of nitrogen in the free state from the silo. This is substantiated by the later investigations, which show that there is a change from the protein to the simler non-protein compounds, but no loss of nitrogen in normal fermentation.

Gain of ether extract in silage fermentation.

Weiske and Schulze (1884) considered that the gain in ether extract was due to the conversion of sugar into lactic and butyric acids, both of which are soluble in ether. This is substantiated by the later investigators. The extent of the increase in ether extract varies with the production of acids.

Changes in crude fibre and ash content in silage fermentation.

Numerous investigators differ as to changes in these constituents. Some report that there are slight increases, others that there are decreases or no change. There is, in all probability, a disappearance of some of the less resistant celluloses. It seems that the differance in results reported might be due mostly to relative changes in composition. In all probability, all changes in ash content are relative rather than absolute, unless there is seepage of juices so samples analyzed are not uniform. Burke (1917) states that

there was no significant change in the mineral constituents due to fermentation.

It can be concluded that the changes involved, in the normal fermentation of silage, are a decrease in nitrogen free extract which consists of sugars and convertible carbo-hydrates, and true protein; with a gain in ether extract and non-protein nitrogen. There is no significant changes in crude fibre and ash. The total losses in dry matter usually vary from 3 to 10 percent.

Some of the chemical changes due to the normal fermentation of silage

No two silages are exactly alike. It is only natural, in view of the complexity of the causative agents in normal fermentation together with the differences in the raw fermenting materials, that there should be variations in the type of silage produced. There are differences from year to year in the kind of silage produced in the same silo on account of variations in internal and external factors. The losses in silage fermentation involve chemical changes. The extent to which these chemical changes occur, depend mainly upon two factors, namely, the presence or absence of air and the maturity of the fodder. There are numerous chemical compounds produced in silage fermentation. The production of acid, with the decrease in sugars and convertible carbo-hydrate materials and the resulting increase in acids are the changes of primary importance.

Sugar - a factor in acid production.

Early investigators, including Short (1888) and Withycombe (1901), reported observations in which they cite the correlation between high moisture and immaturity of the fodder with high acid content. The New York Experiment Station (1892) reported a loss of sugar in silage fermentation. Babcock and Russell (1900) stated that the acid content of silage made from immature corn is higher than in silage made from mature corn. Russell (1908) states that lactic and malic acids might have been derived from the amino acids or carbo-hydrates. The fact, that sugar is the real factor in acid production was overlooked by

these investigators. It is now known that the presence of acids in silage depend upon the sugar content of the ensilaged mass. It was concluded by Clements and Russell (1904), Annett and Russell (1908), Russell (1908), Furuglio and Mayer (1913), Darnell (1916), Swanson and Tague (1917), Lamb (1917), Burke (1917), and Shaw, Wright and Deysher (1921) that sugar disappears during the silage fermentation, generally entirely, sometimes a trace remains.

Dox and Neidig (1914) report that the sugar content of silage juice two days after ensilage as 2.574 percent with a decrease to 1.218 percent twenty-one days later. Lamb (1917) reports the sugar content in 100 cc. juice as 3.139 gms. and in the silage fifteen days later only 0.161 gms sugar remained.

Blish (1921) found the sugar content of sunflower silage in two different localities to be 0.3 percent and 1.6 percent. The silage resulting from the sunflowers having only 0.3 percent turned dark and there were evidences of protein decomposition. It seems very likely that there was not enough sugar present to produce a sufficient quantity of acid to preserve the silage.

Reed and Fitch (1917), report that corn and sorghums make the very best quality of silage because they contain sufficient fermentable carbo-hydrate material to produce enough acid to preserve silage. Sweet corn, however, contains an abundance of sugars and when ensilaged it produces a very sour silage.

It is known that difficulty is experienced in the ensilage of legumes. The legumes are known to be low in sugar and high in protein.

The addition of molasses, cornmeal or any material containing sugar favors a more rapid and plentiful production of acids. The addition of molasses to rape or alfalfa, by Samarani (1914), Lamb and Evvard (1916), Swanson and Tague (1917), Reed and Fitch (1917), and Hunter (1918) resulted in the increase in the acid content of the silage. Blish (1921) found that the addition of corn to sunflowers increased the acid content as compared with sunflower silage.

It is concluded that there is production of acid when fodder normally undergoes fermentation with a decrease, and sometimes an entire disappearance of

sugar.

The production of acid in silage fermentation.

The formation of acids is the important change that takes place in silage fermentation. Sour silage and European sweet silage both contain acid, the degree of acidity being the chief distinguishing difference. It has been shown that sugar is an important factor in acid production. Compactness is also essential so that fermentation will take place under anaerobic conditions.

Gorini (1918) advocates the inoculation of the fodder with lactic ferments so as to insure the early and rapid production of lactic acid, and to keep down butyric acid fermentation. Frazier (1921) and Fred et al (1921) state that inoculation may hasten and intensify the production of certain products in the early stages of fermentation, but later both chemical and bacteriological analyses reveal no marked differences in the kinds of silage produced. In silage investigations in Oregon (1918-20) the inoculation of fodder with Bact. lactis acidi did not prove to be of value.

Several kinds of acid are produced as the result of silage fermentation. Grandeaux (1884) analyzed Goffart's silage and noted the production of volatile and non-volatile acids, which he labelled acetic and lactic, respectively. Lactic is the principle non-volatile acid as is shown in investigations by Russell (1908), Dox and Neidig (1914), Swanson, Calvin and Hungerford (1913) and Neidig (1918). Acetic and propionic are the principle volatile acids as is shown in investigations by Dox and Neidig (1912), Hart and Willaman (1912) and Neidig (1918). The acetic acid constitutes about 80 percent of the total volatile acid. Other acids that have been found in small quantities in silage are formic, butyric, malic, carbonic, succinic and valeric. The presence of butyric acid is indicative of abnormal fermentation.

The amount of acid produced varies according to the sugar content and the compactness of the mass. Short (1888) reports an acidity of 1.26 percent.

Burrill (1889) reports a variation of 0.39 percent to 2.746 percent in acidity for different samples of silage. Kedzie (1889) reports an investigation in which the acid content was 1.26 percent in silage made from immature corn and 0.70 percent in silage made from mature corn. It has been pointed out that immaturity of the fodder and high sugar content of the fodder go together. Esten and Mason (1912) report an acid content of 1.89 percent in silage when the fermentation temperature was 70 degrees F. and only 0.57 percent when the fermentation temperature was down to 40 degrees F. Dox and Neidig (1914) report the total acidity of silage juice as 2.16 percent, comprised of 0.66 percent volatile and 1.5 percent non-volatile. Darnell (1916) concludes that acid production depends on the amount of soluble carbo-hydrates present. Bechdel (1916) found that the total acidity of silage at points away from the silo walls is greater than that along the walls. Barthel (1921) states that the acids are principally lactic and acetic in the best silage and the average $P_H$ is 3.7 in silage of good quality.

The length of time for maximum acid production varies. Esten and Mason cite an individual case in which 1.1 percent acid was present in silage 24 hours old. They state that acids reach their maximum in from one to three weeks. Swanson and Tague (1917) found that most of the acid, in sweet clover and alfalfa silage, is developed in the first 15 days. Sherman and Bechdel (1918) found that total acids increased from 0.51 percent in silage three days old to 5.39 percent after a period of eighty-one days. The ratio of non-volatile to volatile acidity changed from 1 : 5.40 to 1 : 0.71 during this period. Hunter (1921) states that the major production of acid occurs during the first four days of silage fermentation.

A summarization of the above experimental work reveals that fodder normally undergoes fermentation with the production of considerable acid. The factors favoring acid production are the presence of sugar, rigid exclusion of air and proper temperature.

## The factors affecting the preservation of silage

It has been pointed out that in normal fermentation of silage there is a production of acid and an evolution of carbon-dioxide, with an anaerobic condition attained in the silage mass.

### Acid- a factor in silage preservation

Acidity is a desirable characteristic of silage for it is known to be an important factor in its preservation. It is known that acid is normally present in sufficient quantities in silage to properly preserve it. Dox and Neidig (1914) state that silage must have sufficient acidity to insure its keeping, but beyond this point additional acid is neither essential or beneficial. It is known that acid prevents the development of anaerobic putrefying types of bacteria. If the acid is preserved the silage will keep indefinitely. Burrill (1889) states that decomposing types of bacteria work slower in sour material. Other investigators who report on the importance of acid as a preservative in silage are Esten and Mason (1912), Lamb (1917), Hunter and Bushnell (1916) and Reed and Fitch (1917). However, this has been a point of quite common observation.

Lamb (1917) makes a summary statement in which he concludes that in the presence of acid, it is impossible for the bacteria which cause decay to live and work, unless the presence of air should allow the growth of molds, which in turn destroy the acids, and thus allow putrefactive bacteria to thrive.

No mention was made of the part played by yeasts in the destruction of acid.

Gas - a factor in silage preservation.

The kind and amount of gases that are present in the silage mass is another factor in the preservation of silage. It is not known whether carbon-dioxide, as it exists in the silage mass, exerts any inhibitory effect on micro-organism development after the carbon-dioxide content reaches its maximum. Therefore, it seems doubtful that the presence of carbon-dioxide as it exists in the silo, exerts a marked inhibitory effect on the growth of micro-organisms. The factor in the preservation of silage must be the absence of oxygen, rather than the presence of carbon-dioxide, for it is known that the absence of oxygen will prevent the development of all types of aerobic organisms.

Burrill (1889) analyzed the gas fromthe center of three silos, and found 2.3 percent oxygen and 15.2 percent carbon-dioxide for the average of the three silos. Babcock and Russell (1900) found that the free oxygen of the air spaces is very rapidly consumed and replaced by carbon-dioxide which they state is evolved as an end product of respiration of the tissue cells. They state that there is no free oxygen left in the silage mass twenty-four hours after ensilage. They studied the effect of a carbon-dioxide atmosphere on heat production by placing cut corn in an atmosphere of carbon-dioxide gas. They conclude that carbon-dioxide checked micro-organism development, although their experiment was conducted in the absence of oxygen. Dox and Neidig (1914) found 30.7 percent carbon-dioxide and only a trace of oxygen present in silage. Samarani (1922) states, that, in the Italian method of ensilaging hay, the oxygen of the air is transformed in a few hours into carbon-dioxide which may reach 90 percent of the atmosphere in the silage mass.

Dox and Neidig (1914) report a very complete analyses of the oxygen and carbon-dioxide content of three silos. The analyses were made at twenty-four hour intervals covering a period of fourteen days. They found a complete disappearance of oxygen in two of the silos during the first twenty-four hours.

In the other silo there was 1 percent oxygen remaining after the first twenty-four hours which gradually disappeared during the next three days. After that there was only an occasional trace of oxygen present. The maximum carbon dioxide contents were 68 per cent, 58 per cent and 87 percent on the third or fourth days after ensilage. They conclude that carbon-dioxide is formed very rapidly during the first few days, and after reaching a maximum gradually decreases until a minimum of approximately 20 percent of the entire gas is reached. Oxygen disappears almost entirely during the first few days.

The abnormal fermentation of silage

The extent of the losses due to abnormal fermentation of silage are variable. There is always a loss of some silage on the top and down the sides of the silo. Ordinarily, the fodder is altered in the process of normal fermentation so that it will not spoil. However, if air gains entrance to the normal silage, an abnormal fermentation will take place. Abnormal silage can be differentiated from normal silage by being dark brown in color, alkaline in reaction and characterized by yeasty, moldy or putrefactive odors or more often a combination of these odors, with or without noticeable mold spores or mycelium. The causative factors of abnormal fermentation are yeasts, molds and bacteria. They are always present in silage and fodder. If oxygen gains entrance to the mass the yeasts and molds will develop and reduce the acidity. After the acid is destroyed, the putrefying bacteria multiply with the breaking down of the proteins and a liberation of ammonia.

The micro-organism flora of silage and fodder.

It is known that yeast cells are always present on the cut material as it goes into the silo. Yeast functions as an alcoholic ferment under anaerobic conditions. Under aerobic conditions yeast functions like a plant and multiplies, with the reduction of acidity, due to oxidation. Hence, yeasts are a

factor in the abnormal fermentation of silage. Esten and Mason (1912), Hunter and Bushnell (1916), Hunter (1918), and C. A. Hunter (1921) give counts, including corn fodder, corn silage, alfalfa silage, cane fodder silage, and Canada field pea and oat silage, in which the counts vary from 10,000 to 2,000,000. A yeast count of 500,000 is common in silage. Esten and Mason (1912) found seven different types of yeast in silage.

Among the investigators, who have reported on the presence of mold in silage are Burrill (1889), Babcock and Russell (1903), Russell (1908), Mundy (1914), Eckles (1915), Reed and Barber (1917), Robak (1920) and McHargue (1920). Burrill (1889) states that silage, under the decomposing influence of molds, including Mucors, Penicillium and a black mold, will become a homogenous, pasty decayed mass with a bad color. Reed and Barber (1917) found 22 different species of fungi from 15 samples of moldy silage. These fungi were isolated from silage that had been reported as injurious to livestock as well as from silage that was entirely harmless. The above investigators agree that mold will develop in the presence of air and cause an abnormal type of fermentation in silage.

It is known that bacteria, both of acid and alkaline types, are present in large numbers in silage. Burrill (1889), Hunter (1916), C. A. Hunter (1921) and Burri are a few of the investigators who have reported on the presence of liquifying types of bacteria in silage. O. W. Hunter (1916) and C. A. Hunter (1921) gives counts on liquifiers varying from 1,000 to 100,000,000. Burri reports that in 20 samples of green fermenting material bacteria were present up to 1,000,000,000 per gram. He states that there were always present members of the hay and potato Bacillus group. These types are strictly aerobes that are able to grow at high temperatures. They were found mostly in the dark colored and manure-like parts of the silage.

The presence of air - a factor in abnormal fermentation.

It has been known for many centuries that perishables will spoil in the presence of air. Silage is a perishable. Silage is contaminated with large numbers of yeasts, molds and bacteria. Thus, it is evident that the means of preserving silage is to keep the air away from it. Under normal conditions the walls of the silo and the abnormal layer of silage on the top of the silo perform this necessary function. Keeping the air away from silage prevents the growth of the acid destroying organisms, as yeasts and molds. The presence of acid prevents the development of putrefying types of bacteria.

Some of the investigators, who have reported on air being a factor in abnormal fermentation, are Moser (1870), Weiske and Schulze (1884), Henry and Woll (1888), Cooke (1889), Burrill (1889), Babcock and Russell (1900), Short (1888), Russell (1908), Esten and Mason (1912), Eckles (1912), the Kansas Experiment station (1915-16), Lamb (1916), Hunter and Bushnell (1915), the New Mexico Experiment Station (1916-17), Swanson and Tague (1917), Francis and Friedeman (1917), Dowell and Friedeman (1919), Neidig and Vance (1919), Wyant (1920) and Neidig (1921).

Uniform distribution, firm packing, high moisture content and fine cutting of the green fodder are factors contributing to success only in so far that they make exclusion of air more certain.

Russell (1908) reports the differences in types of fermentation when air is present or absent as follows:

| Air excluded (covered and packed tight) | Air admitted (loose, not covered) |
|---|---|
| Normal silage formed | Putrefaction |
| No mold | Mold |
| Some loss in dry matter | Considerable loss in dry matter |
| Acid in reaction | Alkaline in reaction |
| Acetic acid produced | No acid formed |
| Protein changes to non-protein | Non-protein changes to protein |
| No loss of nitrogen | Nitrogen lost |

Eckles (1912) considered that silage spoils in two ways, namely, a rotting which causes a humus like mass to develop and a molding. They both develop in the presence of air.

Lamb (1916), in a summary statement, concludes that changes which are normal to the formation of good silage take place almost entirely in the absence of air. The oxygen of the silo is used up early in the process of fermentation. From this point the presence of oxygen is fatal to the proper preservation of the silage, because air permits the development of molds, which quickly destroy the acids and thus allows the silage to spoil.

The relation of moisture content of silage to abnormal fermentation.

Lack of sufficient moisture is the most common cause of air being present. Numerous investigators have made moisture determinations on silage but information on the condition of the silage has not been reported. The presence of mold has been noted when the moisture content of the fodder was low and has a dry appearance.

Hunter and Bushnell (1917) state that a moisture content of 70 percent gives a very favorable condition for active fermentation while a low moisture content will give abnormal conditions for fermentation. Blish (1921) advises not to allow the moisture content of sunflower silage to drop lower than 65-70 percent. The Oregon Station (1918-20) reports that the average corn silage has 26.1 percent dry matter and 73.9 percent moisture.

The review of literature reveals that there has never been a detailed investigation made, with the view of correlating moisture content of silage with abnormal fermentation of silage.

Heat production in abnormal fermentation.

It is a well known fact that when a mass of wet organic matter is placed in a heap there is evolution of heat. The heat developed in fermentation cannot be entirely dissipated and remains to raise the temperature of the mass.

Much confusion arose among the early investigators as to the temperature in normal fermentation. The temperature readings were made at the surface and it was quite generally assumed that the temperature of the whole mass was high. A former hypothesis to explain why silage keeps was that the fermentation set up by micro-organisms evolved so much heat that everything is killed and the mass becomes sterile.

Burrill (1889) states that the evolution of high degrees of heat is not the result of alcoholic, acetic or ammoniacal fermentation, but is the same in all particulars as that taking place in hot stable manure, which is due to two or more species of rod-like Bacilli, which appear to cause butyric fermentation. He reports a maximum temperature of 158 degrees F. He cites the process recommended by Fry and Voelker in England and Miles in the United States for making sweet ensilage. This process consists of filling slowly for exposure of the mass to the air. The fodder becomes very hot and it was thought that the heat destroys the acid fermenting bacteria. He states that this hot fermentation is not due to yeasts for no species of yeasts can retain their activity at 140 degrees F., which is not an uncommon temperature for hot ensilage. He further states that yeast loses its power of growth and development in an accellerating ratio as the heat increases above 30 degrees C.

Maximum temperatures in abnormal fermentation are reported by Short (1888), Woll (1889), Babcock and Russell (1900), Lamson (1900), Pernot (1902), Esten and Mason (1912), Beach (1912), Eckles (1914), Magruder (1914), Oshel (1915) Hunter and Bushnell (1916), Lamb (1917) and Friedeman (1917). The temperature ranges from 110 degrees to 163 degrees F. as reported by these investigators. A temperature of 130 degrees F. is common in abnormal fermentation. The maximum high temperature in abnormal fermentation may be reached in 3 to 7 days and the mass remains hot for an indefinite period of time. The temperature of normal fermentation, as noted by these same investigators and others, varies from 65

degrees to 100 degrees F. A temperature of 75 degrees to 85 degrees F. is common, in normal fermentation.

Esten (1910) states that high temperatures are found in silage only when the surface is exposed to air and where an alkaline fermentation is in progress, or where the percent of moisture is relatively low.

Eckles (1914) states that the temperature rarely exceeds 100 degrees F. unless the silage is deficient in moisture or air is present. The growth of mold was recognized as a causative factor of high temperatures.

The chemical composition of abnormal silage

Abnormal fermentation is characterized by an oxidation of the organic acids with production of heat. Molds, yeasts and bacteria are the causative factors of heat production in abnormal fermentation.

Darnell (1916) concludes that mold reduces, for a short time, the amount of each constituent in corn silage, after which there is an increase in crude fibre.

Robak (1920) concludes that the growth of mold causes a consumption of oil with an increase in the free acids and unsaponifiable constituents, with a decrease in the percent of volatile, insoluble and unsaturated acids.

McHargue (1920) states that molds will cause a rapid deterioration of oil, sugar and starch contained in the germs of corn.

Friedeman (1917) found upon analyses of normal and spoiled Darso, ribbon cane, orange cane and sudan grass that the nitrogen content of spoiled silage is higher than that of normal silage and accounts for this difference in composition as being due to the action of aerobic ammonifying or nitrifying types of micro-organisms. The ash content of spoiled silage is higher relatively than that of normal silage showing that destruction of organic matter has taken place. The percent of the water soluble acids is considerably lower in spoiled silage than in normal silage. He noted that in one sample of spoiled cane silage that 89.32 percent of the volatile acids was acetic, 9.25 percent formic and 1.43 percent

butyric. He states, but gives no analyses, that the percent of fibre, nitrogen free extract and ether extract are only slightly lower in spoiled silage as compared with normal silage.

Hunter (1918) and Lavenir and Chaudet (1920) found upon chemical analyses of alfalfa silage that there is protein decomposition, as evidenced by an abnormally high content of non-protein nitrogen and ammonia. The non-protein nitrogen consisted almost entirely of amino acids.

Barthel (1921) states that the presence of butyric acid is indicative of putrefactive fermentation. He found that poor silage had the largest percent of nitrogen in the form of ammonia and in good silage the amount of nitrogen in peptone and amino acids varies from 22.8 to 45.3 percent of the total. A poor grade of silage from a pit silo had a PH value of 4.55 which is higher than normal.

Neidig (1921) found, upon analyses of sugar beet top silage, that six samples out of seven had some butyric acid present. He states that the presence of butyric acid indicates an abnormal fermentation resulting in partial decomposition of the silage.

In summarizing, it will be stated that silage undergoes a change in abnormal fermentation. The extent of these changes varies according to the type of abnormal fermentation. As compared to normal silage, there is a decrease in acids, nitrogen-free extract, and true protein with possibly a slight decrease in ether extract and crude fibre. With an increase in total nitrogen, non-protein nitrogen, $P_H$ value, ammonia and ash. The increase in ash is, in all probability, only relative. The presence of butyric acid is indicative of abnormal fermentation.

## EXPERIMENTAL

The experimental work which follows is divided into five parts. The purpose of these five experiments was to study the following: (1) The role that acid plays in the preservation of silage; (2) The relation between the moisture content of silage and the growth of mold; (3) The changes in flora of micro-organisms, temperature and hydrogen ion concentration when normal silage undergoes abnormal fermentation; (4) the causative factors in abnormal fermentation of silage; and (5) the chemical changes in the abnormal fermentation of silage.

### Experiment I

The review of literature reveals the fact that there are a large number of micro-organisms of various kinds present in silage, i.e. molds, yeasts and bacteria. In view of this complexity of biological factors, it is apparent that the method of controlling the fermentation and preservation of silage is by the regulation of the conditions that are favorable or unfavorable to the development of these organisms, rather than the elimination of the causative agents.

Object of Experiment:

To substantiate the fact that acid is a factor in silage preservation.

Plan of Experiment.

1. The isolation of alkaline producing types of bacteria and the inoculation of some of them into sterilized acid silage.

2. The isolation of yeasts and molds and inoculation of them into acid silage alone and with alkaline producing types of bacteria.

Experimental data

A sample of abnormal sunflower silage was plated out on lactose agar (brom cresol purple added as indicator). From this plating twenty-nine colonies were picked and transferred to lactose agar slants. Nine of these cultures were

used for further study, of which a description follows:

| Number of culture | Morphology | Reaction produced on media | Growth in broth |
|---|---|---|---|
| 4 | Medium rods | Alkaline | Pellicle |
| 8 | Very short rods | " | Cloudy, sediment |
| 14 | Medium heavy rods | " | Pellicle |
| 20 | Short rods | " | Cloudy, sediment |
| 23 | Cocci | - | Cloudy, sediment |
| 25 | Long rods | Alkaline | Sl. floc. Growth and sediment |
| 26 | Long rods | Alkaline | Sl. floc. Growth and sediment |
| 27 | Short rods | Alkaline | Cloudy, sediment |
| 28 | Short rods | Alkaline | Sl. pellicle, cloudy, sediment |

No attempt was made to identify the organisms.

These nine organisms were inoculated into sterilized acid silage, (in half pint bottles) and incubated at about 37 degrees C., The sterile silage was acid to brom cresol purple indicator. Eleven days later all lots appeared unchanged and were acid to brom cresol purple. Then four yeast cultures in dextrose broth were added to numbers 4, 8, 20 and 25. On the following day there was noticeable growth in these four. In three days they gave an alkaline reaction with brom cresol purple. After ten days slides were made which showed abundant yeast cells but the bacteria could not be found in abundance. Plates made later showed the presence of bacteria. After twenty-seven days had elapsed all nine samples were tested for reaction. The four samples that had yeast added were alkaline and the other five samples with bacteria alone were acid to brom cresol purple.

Two samples of abnormal corn silage, one of which was heating at the time and the other sample in close proximity to the heating silage, were plated out. They were both alkaline to brom cresol purple. From the plates, six alkaline and seventeen acid producing organisms were isolated.

The following is a description of the alkaline producers:

| Number of culture | Morphology | Reaction produced on media | Growth in broth |
|---|---|---|---|
| 3Hc | Long rods | Alkaline | Pellicle |
| 3Hd | Long rods | " | Sl. sediment |
| 6Hc | Medium rods | " | Pellicle |
| 6Hd | Medium rods | " | Heavy red pellicle |
| 6Uf | Very short rods | " | Pellicle |
| 6Ug | Medium rods | " | Pellicle |

These twenty-three organisms were inoculated into sterile acid silage, in test tubes and incubated two days at 37 degrees C. and then at room temperature. Eighteen days later they were tested for reaction with methyl red and brom phenol blue. They were all acid, including a check tube to methyl red and a slight acidity to brom phenol blue, which demonstrated that the organisms did not reduce the acidity.

This was followed by plating out two more samples of abnormal corn silage and colonies were picked. There was a resemblance in flora in both samples, one of which was decayed, and the other a dry moldy sample. As before, the flora was varied. The organisms isolated are described briefly as follows:-

No. 6A1 - Medium heavy rods in chains, spores
" 12-2 - Medium long rods
" 11-3 - Medium light rods, spores
" 12-4 - Medium rods, spores.

These organisms, plus a few others isolated before were inoculated into sterile acid silage in the following combinations with the results as indicated:

6uf plus white mold - a strong putrefactive ammonia odor;

Strongly alkaline to brom cresol purple in four days.

11-3 plus a mold that liquifies gelatin - a putrefactive odor in eight days;

Alkaline to brom cresol purple.

12-4 plus grey mold - A slight putrefactive odor in fourteen days;

Acid to brom cresol purple

12-2 plus yeast - acid to brom cresol purple in fourteen days. A fishy odor after twenty-three days.

6A1 plus yeast - acid to brom cresol purple after fourteen days.

6Hd plus yeast - acid to brom cresol purple after fourteen days. A putrefactive odor after twenty-three days.

6Ug plus yeast - acid to brom cresol purple after fourteen days. A putrefactive odor after twenty-three days.

The organisms 6Uf and 11-3 were inoculated into practically neutral sterile silage, and putrefactive odors developed in three and six days respectively.

Discussion of results.

The above experiment indicates that acid is an important factor in the preservation of silage. The quantity of acids normally present in corn silage is sufficient to inhibit the growth of alkaline or putrefying types of bacteria. Both yeasts and molds reduce the acidity in the presence of air and after the acid is reduced the putrefying types will develop and produce an alkaline silage with the liberation of free ammonia. Hence, in the production of silage, acid development is desirable for it acts as a preservative and as long as acid destroying organisms, which require the presence of air for development, are kept from developing the silage will keep. A formula for silage preservation is:

Acid plus lack of air = preservation

## Experiment II

### The relation between moisture content of silage and growth of mold

It has been believed by farmers, extension men and others for many years, that the cause of moldy silage is a lack of sufficient moisture in the material as put into the silo and that this moldy condition may be prevented by putting the crop into the silo in a greener condition or by adding water at the time of filling. It is known that a large amount of water tends to increase the compactness of the ensilaged material and is a factor in the exclusion of air. Mold growth occurs only in the presence of air. Consequently, it would seem that a relation exists between moisture content and growth of mold.

Object of experiment.

To correlate moisture content of silage with the growth of mold for the purpose of ascertaining whether moisture content is the important factor in the presence or absence of mold growth.

Method of gathering information.

The system of gathering information involved the survey method. By this method there is always considerable guesswork and approximation. However, it has been shown in statistical work that if enough information is obtained the average will approximate accuracy. The official test supervisors employed by the University of Minnesota gathered silage samples and filled out special information blanks in cooperation with the farmers. An example of the information blank used with typical information received is shown on the following page.

Plan of experiment.

Paraffined lined containers and information blanks were sent to official test supervisors employed by the University of Minnesota in different parts of the State of Minnesota. The silage samples were weighed immediately upon arrival, and air dried. They were weighed at five day intervals, usually for

## Directions for sampling silage

Object: The object of getting these samples is to gather information regarding the conditions that accompany the molding of silage.

Moldy silage not wanted: When mold appears in parts of the silo do not take the sample from the moldy part, but from typical silage that has not molded. Be careful and give full information about the mold.

Taking the sample: With the small amount taken unusual care must be given to getting a fair sample. If the sample is taken in the silo throw back the silage for a depth of 4 or 5 inches in not less than four places and fill the mailing case by taking some from each spot. If the sample is taken from silage as thrown out for feeding, take a hand full at a time from different spots. Take it as it comes without leaving out or putting in any extra corn or pieces of stalks. Pack the silage tightly in the case. Mail the sample at first opportunity.

## Information to be secured from owner

1. Owner of farm - - Address Shakopee, Minnesota
2. Kind of silo Hollow cement block Size 16 x 42
3. Condition of wall inside Good; cement whitewashed every three years.
4. Any trouble from mold this year Yes
5. Any trouble in previous years No
6. Stage of maturity of corn when cut Just ripe but stalks were too dry on account of dry soil
7. How long did corn lie on ground before being hauled in? 3 hours
8. Was corn frosted or lodged? No
9. How many men were used in tramping? Two big fellows
10. Was water added at time of filling? No, but should have
11. If mold is present when was it first noticed?
12. In what part of the silo does it occur? In center
13. Color of mold Greyish white
14. Information secured by - - Date March 8th, 1922
15. Additional information The men tramped around the outside only, thinking that the center would pack itself.

three more weighings, until a constant weight was secured. Allthe moisture determinations were made and calculated on the air dry basis. Of the total number of weighings for each sample, the weighing which showed the least amount of dry matter was used. The dry matter content is calculated on the percent basis by computation from the original weighing which equals 100 percent. The percent moisture is calculated by difference. All the samples were examined for maturity, fineness of cutting, presence of mold, odors, moist or dry appearance and general condition.

Discussion of experimental data.

The study of the blanks reveal that there are numerous factors which contribute to the moisture content of silage, such as maturity of corn, length of time corn was on ground before hauling and the addition of water at the time of filling. The only means of correlating these factors, as to their relation to mold growth, is to group them into one factor, namely, moisture content and then correlate this one factor with the growth of mold.

The examination of the silage revealed the fact that silages are different as to odor and flavor. These various odors were noted:

For normal silage - acid of varying degrees, vinegary, weedy, fruity, beer-like, bitter, pungent and apple odors

For abnormal fermentation - yeasty, moldy, caramelized, and putrefactive odors.

A correlation between number of men used in tramping with the presence or absence of mold revealed that, with a total of 63 reporting on this point, 28 percent of the farmers who used two men or less in the silo had mold in their silage, while only 13 percent of the farmers who used three men or more in the silo had mold. Whether this relationship is merely an incident or is really a factor in the molding of silage is a question.

The following table gives the moisture and dry matter contents on seventy-one samples of silage, classified into four groups according to growth and location of mold. In this table, reference to the presence of mold means that mold growth is apparent to the naked eye.

TABLE I

Summary showing relation between growth of mold and moisture content

| Number of sample | Initial Wt. of silage | Wt. of air dried matter | % air dried matter | % moisture (by difference) |
|---|---|---|---|---|
| | grams | grams | % | % |
| MOLD PRESENT | | | | |
| 34 | 193.5 | 68.0 | 35.15 | 64.85 |
| 37 | 339.0 | 141.5 | 41.44 | 58.56 |
| 40 | 159.5 | 72.0 | 45.15 | 54.85 |
| 60 | 176.0 | 66.0 | 37.50 | 62.50 |
| 61 | 163.0 | 77.0 | 47.24 | 52.76 |
| 62 | 276.0 | 93.5 | 33.88 | 66.12 |
| 70 | 139.0 | 54.5 | 39.21 | 60.79 |
| 79 | 196.0 | 78.5 | 40.05 | 59.95 |
| 91 | 134.5 | 56.0 | 41.64 | 58.36 |
| 109 | 216.0 | 77.0 | 35.65 | 64.35 |
| Average of 10 samples | | | 39.69 | 60.31 |
| MOLD AT WALLS** | | | | |
| 41 | 207.0 | 81.5 | 39.37 | 60.63 |
| 52 | 145.0 | 50.0 | 34.50 | 65.50 |
| 54 | 219.0 | 81.5 | 37.20 | 62.80 |
| 66 | 229.5 | 80.0 | 34.86 | 65.14 |
| 84 | 255.0 | 80.5 | 31.57 | 68.43 |
| 85 | 172.0 | 55.0 | 32.00 | 68.00 |
| 86 | 269.0 | 93.5 | 34.76 | 65.24 |
| 89 | 204.5 | 54.5 | 26.65 | 73.35 |
| 90 | 291.0 | 106.5 | 36.60 | 63.40 |
| 93 | 174.0 | 51.5 | 29.60 | 70.40 |
| 94 | 222.0 | 83.5 | 37.61 | 62.39 |
| 96 | 282.0 | 103.0 | 36.53 | 63.47 |
| 105 | 191.5 | 77.5 | 40.47 | 59.53 |
| 107 | 260.5 | 87.0 | 33.40 | 66.60 |
| 111 | 224.0 | 59.0 | 26.34 | 73.66 |
| Average of 15 samples | | | 34.09 | 65.91 |
| MOLD IN SPOTS | | | | |
| 36 | 185.5 | 67.0 | 36.12 | 63.88 |
| 44 | 129.5 | 49.5 | 38.23 | 61.77 |
| 47 | 271.0 | 69.5 | 25.64 | 74.36 |
| 59 | 235.5 | 89.5 | 38.00 | 62.00 |
| 87 | 213.0 | 78.0 | 36.62 | 63.38 |
| Average of 5 samples | | | 34.92 | 65.08 |

Table I (continued)

| Number of sample | Initial Wt. of silage | Wt. of air dried matter | % air dried matter | % moisture (by difference) |
|---|---|---|---|---|
| | grams | grams | % | % |
| NO MOLD PRESENT | | | | |
| 35 | 300.0 | 65.0 | 21.67 | 78.33 |
| 45 | 137.0 | 39.0 | 28.47 | 71.53 |
| 46 | 140.0 | 40.5 | 28.90 | 71.10 |
| 48 | 211.0 | 61.5 | 29.15 | 70.85 |
| 49 | 412.0 | 119.0 | 28.88 | 71.12 |
| 50 | 210.0 | 68.0 | 32.38 | 67.62 |
| 51 | 423.5 | 146.5 | 34.59 | 65.41 |
| 53 | 160.5 | 69.5 | 43.30 | 56.70 |
| 55 | 478.0 | 130.0 | 27.20 | 72.80 |
| 57 | 178.5 | 49.5 | 27.73 | 72.27 |
| 58 | 150.5 | 60.5 | 40.33 | 59.67 |
| 63 | 177.0 | 60.0 | 33.90 | 66.10 |
| 64 | 170.0 | 54.0 | 31.77 | 68.23 |
| 65 | 211.5 | 88.0 | 41.61 | 58.39 |
| 67 | 264.0 | 82.0 | 31.07 | 68.93 |
| 68 | 146.0 | 45.0 | 30.83 | 69.17 |
| 69 | 171.0 | 65.5 | 38.30 | 61.70 |
| 71 | 184.0 | 47.5 | 25.82 | 74.18 |
| 72 | 270.0 | 78.5 | 29.08 | 70.92 |
| 73 | 229.5 | 71.0 | 30.94 | 69.06 |
| 74 | 237.5 | 75.0 | 31.58 | 68.42 |
| 75 | 238.5 | 62.0 | 26.00 | 74.00 |
| 77 | 318.5 | 82.0 | 25.75 | 74.25* |
| 78 | 326.0 | 89.0 | 27.30 | 72.70* |
| 81 | 277.0 | 80.5 | 29.06 | 70.94 |
| 82 | 263.0 | 82.0 | 31.19 | 68.81 |
| 83 | 298.0 | 91.0 | 30.54 | 69.46 |
| 88 | 201.5 | 61.0 | 30.27 | 69.73 |
| 92 | 279.0 | 93.5 | 33.51 | 66.49 |
| 95 | 183.0 | 49.0 | 26.78 | 73.22 |
| 97 | 339.0 | 107.0 | 31.56 | 68.44 |
| 98 | 267.0 | 76.0 | 28.47 | 71.53 |
| 99 | 141.0 | 50.0 | 35.46 | 64.54 |
| 100 | 261.0 | 65.5 | 25.10 | 74.90* |
| 102 | 216.0 | 68.0 | 31.48 | 68.52 |
| 104 | 194.0 | 71.0 | 36.60 | 63.40 |
| 106 | 282.0 | 87.0 | 30.85 | 69.15 |
| 108 | 243.0 | 76.5 | 31.48 | 68.52 |
| 113 | 300.0 | 86.0 | 28.67 | 71.33 |
| 114 | 300.0 | 87.8 | 29.25 | 70.75 |
| 115 | 300.0 | 73.8 | 24.58 | 75.42 |
| Average of 41 samples | | | 30.76 | 69.24 |

* Sunflower silage

** This class includes also mold around doors and mold to a greater depth on top than is normal.

Discussion of Table

The table indicates that a relation exists between the moisture content of silage and the presence or absence of mold. The average moisture is 8.93 percent higher in the forty-one samples of silage having no mold than in the ten samples with mold present. The silage with mold present had in all but one case a moisture content of less than 65 percent. The silage with no mold present had in all but 6 cases a moisture content of more than 65 percent. The silage with mold at walls and doors varied in moisture content from 73.66 percent to 59.53 percent, a difference of 14.13 percent. The average percent moisture of 41 samples having no mold present was 69.24 percent.

Discussion of results.

The moisture content is a factor in the preservation of silage only in so far as it contributes to compactness, that is, in the exclusion of air. It is known that the exclusion of air is essential because mold spores, which are invariably present in silage, require the presence of air for their development.

The interpretation of the figures in the table indicate that a high moisture content contributes to the elimination of air. In the group where mold was present, the lack of moisture made the elimination of all air impossible, making the conditions favorable for mold growth. In the group where mold was present in spots, it would seem that air was enclosed in the ensilaged material at this particular spot. The presence of air in these limited portions might be due to faulty packing and settling, or to low moisture content or to a combination of both of these. In the group where mold is present at walls and doors, there is a wide variation in the moisture content which would indicate that silage will mold when the moisture content is high or low, if exposed to air. In the group free from mold, there is, with few exceptions, a high moisture content of the silage. However, a few samples in this group have a low moisture content. This low moisture content with no mold can be explained by the fact

that these samples had mature kernels present, which tend to increase the weight of a unit volume, and were cut more finely which makes compactness more certain.

It can be concluded that the moisture content of silage is an important factor in determining whether mold will be present or absent.

## Experiment III

It is known that when silage undergoes abnormal fermentation there is a reduction in acidity, with an increase in temperature due to oxidation. When these abnormal changes in silage continue, the mass of organic matter ultimately takes on a black, humus-like appearance. The question arising, from the scientific point of view, is what are the causative factors of these changes and, from a practical point of view, how can these abnormal changes be prevented so as not to involve more than a necessary loss. It is recognized that silage will remain normal if sufficient acidity is produced and then preserved by keeping destructive organisms, which require the presence of air, from developing. However, the causes of abnormal fermentation of silage and the chemical products produced are not so well known.

Object of experiment.

To study the changes in flora, temperature and hydrogen ion concentration when normal silage or green fodder undergoes abnormal fermentation.

Plan of experiment.

This experiment may conveniently be divided into three parts, as follows:

Part I of Experiment III

Preliminary experiment.

Two samples of normal silage were placed in an insulated box and temperature readings, supplemented by a few plate counts and reaction determinations were made at different times on the silage as it changed from normal to abnormal, due to destructive fermentation process that takes place in the presence of air.

Part II of Experiment III

Detailed experiment similar to preliminary experiment.

About twenty pounds of normal silage from the Dairy, Veterinary and Beef barn silos were placed separately into three small paraffin-lined churns which had been placed into a long box and insulated with shavings and saw dust. All counts of organisms were made by the standard plate method, using lactose agar for bacteria and plain agar plus dextrose tartaric acid for yeasts and molds. Five grams of silage plus forty-five cubic centimeters of sterile water was ground up with a sterile mortar and pestle. One cubic centimeter of this silage solution added to ninety-nine cubic centimeters sterile water gave a 1:1000 dilution representing one gram of silage. All temperatures were taken with an ordinary dairy thermometer. The initial and final hydrogen ion concentrations were made with a potentiometer and all the other hydrogen ion concentrations, colorimetrically.

Part III of Experiment III

Supplementary experiment, similar to, but not as detailed as, Part 2, for the purpose of determining if the changes which occur in the top layers of silage in large silos are similar to those occurring when normal silage undergoes abnormal fermentation.

Experimental data

Part I of Experiment III

The two samples of normal silage that went through the process of abnormal fermentation changed from acid to alkaline in reaction, as was indicated by brom cresol purple. On plating, sample 1 showed numerous yeast colonies which grew on plates and appeared to be in almost pure culture. There was an absence of mold growth. In sample 2 there were more mold colonies with fewer yeast cells.

Table II gives the temperature changes in the abnormal fermentation of these two samples.

TABLE II

Temperature changes in abnormal fermentation

| Age of sample* | Temperature | |
|---|---|---|
| | Sample #1 | Sample #2 |
| | F | F |
| 0 days | 70.0 | 72.0 |
| 1 " | - | 80.0 |
| 2 " | 78.0 | - |
| 3 " 8 a.m. | 90.0 | 76.0 |
| 3 " 10 a.m. | 100.0 | - |
| 3 " 5 p.m. | 107.0 | - |
| 4 " | 101.0 | 78.0 |
| 5 " | 98.0 | 84.0 |
| 6 " | 98.0 | 89.0 |
| 7 " | - | 83.0 |
| 8 " | 100.0 | - |
| 9 " | 97.0 | - |

*The time elapsed after putting the normal silage into the container.

This preliminary experiment indicated that yeasts are a factor in producing a rise in temperature and a reduction in acidity in abnormal silage fermentation.

Part II of Experiment III.

The three samples of normal silage that went through the process of abnormal fermentation changed very markedly in flora, reaction and appearance.

On plating, the predominating colonies always are alkaline with a strong ammonia odor showing that the alkaline producing types of bacteria predominate. On the higher dilution plates there were a few acid colonies. There were many spreaders which interferred with the counting of colonies. The bacterial flora seemed to be constant throughout the abnormal fermentation with a marked increase in numbers. There appeared to be at least four different types of mold present. The yeast colonies were large on the surface and small underneath the surface of the agar.

Table III shows the changes in numbers of bacteria, yeasts and molds as the process of abnormal fermentation progressed.

Table IV gives the changes in temperature in the abnormal fermentation.

Table V shows the changes in reaction from acid to alkaline, when normal silage goes through the process of abnormal fermentation as shown by use of hydrogen ion indicators.

The changes in temperature, H ion concentration, and yeast and mold counts in samples 1, 2, 3 of Part II, Experiment III, were plotted. They are shown in Graphs I, II and III respectively.

TABLE III

Changes in flora in abnormal fermentation

| Date | Sample #1 | Sample #2 | Sample #3 |
|---|---|---|---|
| | Per Gram | Per Gram | Per Gram |
| Bacteria | | | |
| April 22 | 5,850,000 | 10,000 | 3,000 |
| April 25 | - | 2,510,000 | 23,000 |
| April 27 | 246 , 000,000 | 128,000,000 | - |
| April 29 | 600,000,000 | 83,000,000 | 45,000,000 |
| May 1 | 550,000,000 | 26,000,000 | 400,000,000 |
| May 3 | 1,200,000,000 | 950,000,000 | 400,000,000 |
| May 6 | 172,000,000 | 800,000,000 | 256,000,000 |
| May 15 | 800,000,000 | 1,100,000,000 | 1,100,000,000 |
| Yeasts | | | |
| April 22 | 432,000 | 0 | 100 |
| April 25 | 2,280,000 | 890,000 | 200 |
| April 27 | 15,700,000 | 6,200,000 | - |
| April 29 | 4,000 | 2,000,000 | 900,000 |
| May 1 | 0 | 1,100,000 | 1,075,000 |
| May 3 | 0 | 21,000 | 16,000 |
| May 6 | 0 | 0 | 0 |
| May 15 | 0 | 0 | 310,000 |
| Molds | | | |
| April 22 | 10,000 | 0 | 0 |
| April 25 | 0 | 0 | 100 |
| April 27 | 0 | 0 | - |
| April 29 | 0 | 0 | 0 |
| May 1 | 45,000 | 1,200,000 | 1,600,000 |
| May 3 | 302,000 | 2,100,000 | 5,800,000 |
| May 6 | 200,000 | 600,000 | 14,800,000 |
| May 15 | 8,500,000 | 4,100,000 | 17,000,000 |

TABLE IV

The Relation of abnormal fermentation to temperature

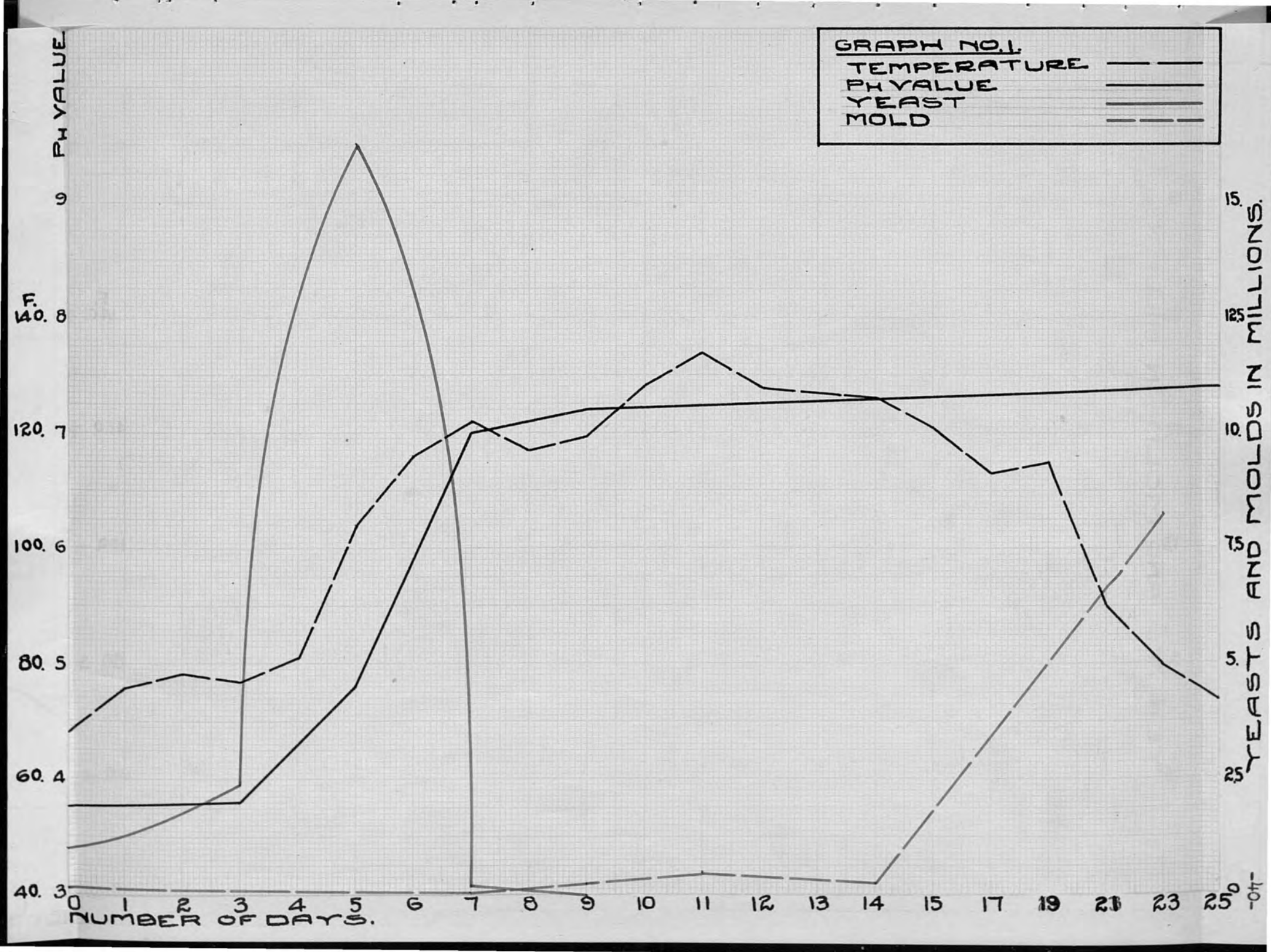
| Date | | | Room Temperature | Sample #1 | Sample #2 | Sample #3 |
|---|---|---|---|---|---|---|
| | | | degrees F. | degrees F. | degrees F. | degrees F. |
| April | 20 | a.m. | 60 | 45 | 45 | 45 |
| | 21 | a.m. | 66 | 61 | 59 | 62 |
| | 22 | a.m. | 63 | 65 | 62 | 62 |
| | | p.m. | 74 | 69 | 64 | 65 |
| | 23 | a.m. | 66 | 76 | 66 | 64 |
| | 24 | a.m. | 64 | 78 | 67 | 65 |
| | 25 | a.m. | 58 | 77 | 69 | 66 |
| | 26 | a.m. | 62 | 76 | 76 | 64 |
| | | p.m. | 67 | 86 | 93 | 65 |
| | 27 | a.m. | 58 | 102 | 109 | 70 |
| | | a.m. | 66 | 104 | 101 | 72 |
| | | p.m. | 70 | 107 | 99 | 75 |
| | 28 | a.m. | 61 | 112 | 109 | 85 |
| | | p.m. | 73 | 120 | 107 | 95 |
| | 29 | a.m. | 62 | 122 | 107 | 99 |
| | | p.m. | 74 | 121 | 109 | 112 |
| | 30 | p.m. | 72 | 117 | 110 | 104 |
| May | 1 | a.m. | 64 | 119 | 112 | 105 |
| | | p.m. | 74 | 113 | 112 | 105 |
| | 2 | a.m. | 69 | 125 | 120 | 119 |
| | | p.m. | 71 | 130 | 128 | 122 |
| | 3 | a.m. | 70 | 134 | 127 | 121 |
| | | p.m. | 78 | 132 | 125 | 120 |
| | 4 | a.m. | 68 | 128 | 122 | 125 |
| | 5 | a.m. | 69 | 127 | 130 | 129 |
| | 6 | a.m. | 74 | 126 | 123 | 126 |
| | 7 | a.m. | 75 | 121 | 122 | 124 |
| | 8 | a.m. | 71 | 113 | 125 | 122 |
| | 9 | a.m. | 71 | 113 | 122 | 120 |
| | 10 | a.m. | 78 | 112 | 121 | 121 |
| | 11 | a.m. | 81 | 117 | 117 | 122 |
| | 12 | a.m. | 67 | 112 | 116 | 111 |
| | 13 | a.m. | 71 | 90 | 111 | 92 |
| | 15 | a.m. | 63 | 80 | 90 | 79 |
| | 16 | a.m. | 65 | 80 | 84 | 79 |
| | 17 | a.m. | 66 | 74 | 76 | 75 |
| | 18 | a.m. | 62 | 71 | 72 | 70 |

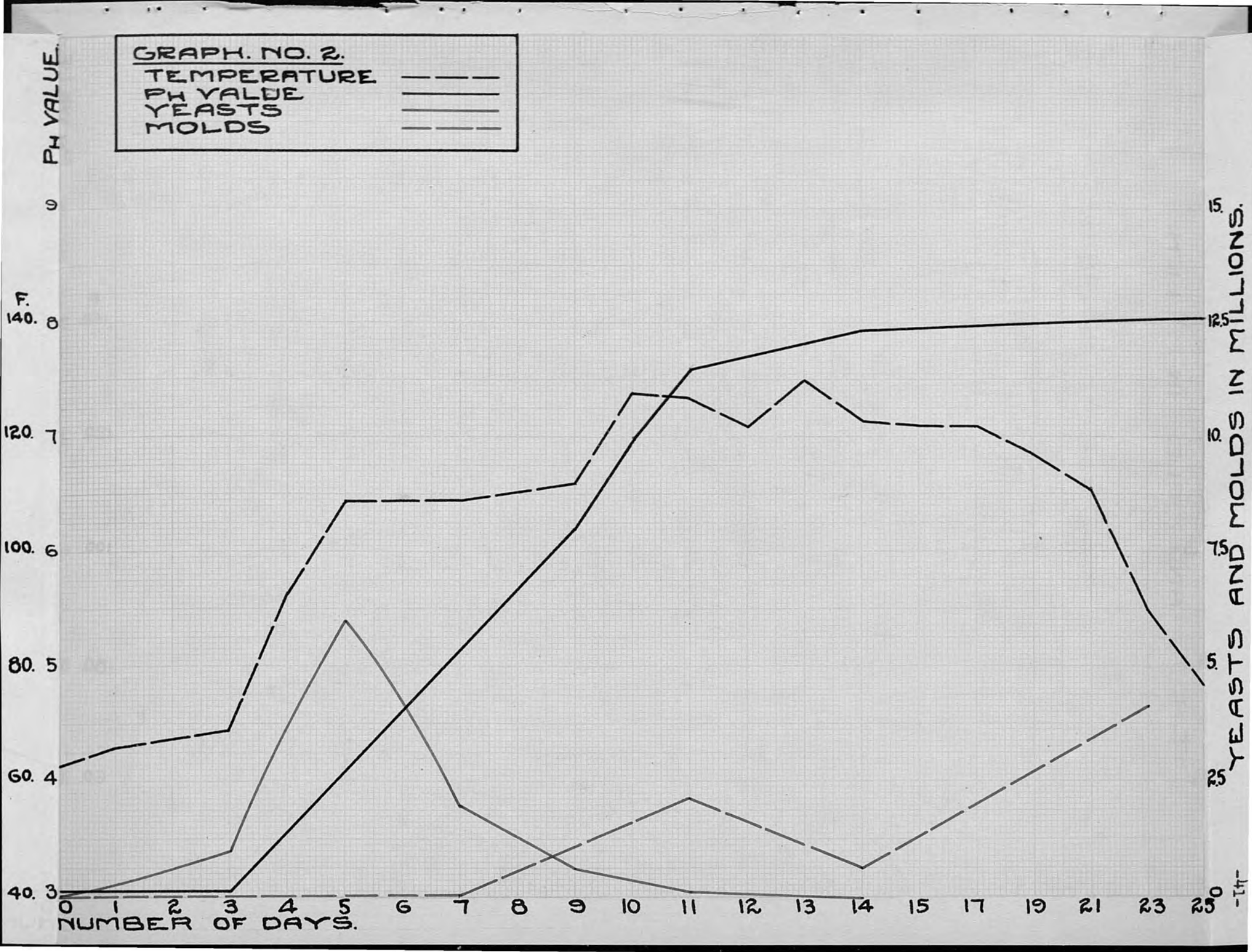
TABLE V

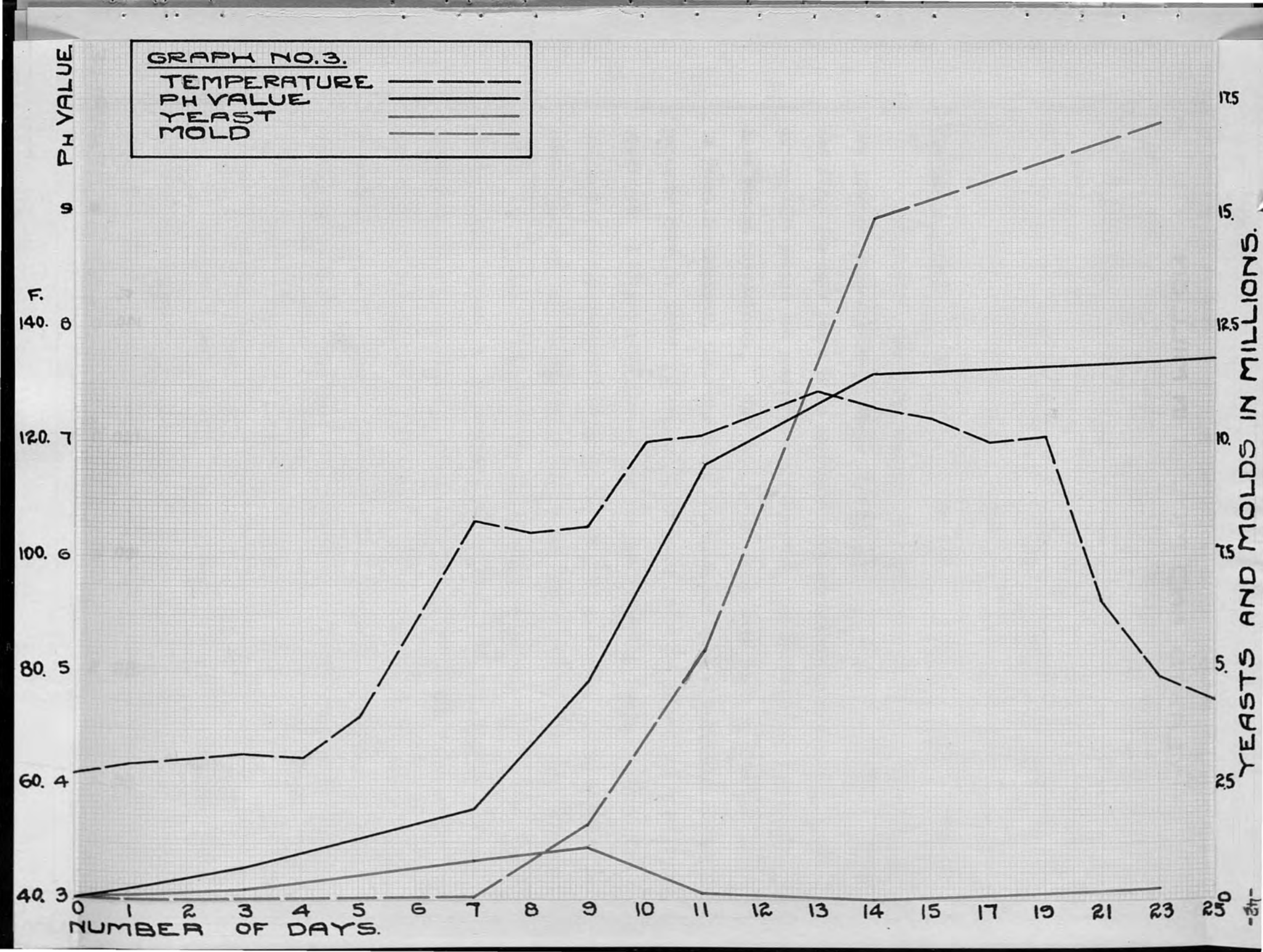
Change in reaction as shown by Hydrogen ion indicators in abnormal fermentation.

| Indicator used | Date | Sample #1 | Sample #2 | Sample #3 |
|---|---|---|---|---|
| Brom phenol blue (yellow - blue) 3.0 - 4.6 | April 20 | blue | greenish | slight yellow |
| | April 25 | blue | light blue | light blue |
| | April 27 | dark blue | light blue | - |
| | April 29 | - | dark blue | blue |
| Methyl red (red - yellow) 4.4 - 6.0 | April 20 | red | red | red |
| | April 25 | red | red | red |
| | April 27 | brownish | red | - |
| | April 29 | yellow | brown | red |
| | May 1 | - | yellow | brownish red |
| Brom cresol purple (yellow - purple) 5.2 - 6.8 | April 25 | yellow | yellow | yellow |
| | April 27 | yellow | yellow | - |
| | April 29 | purple | yellow | yellow |
| | May 1 | - | light purple | yellow |
| | May 2 | - | purple | purple |
| | May 3 | - | purple | purple |
| Brom thymol blue (yellow - blue) 6.0 - 7.6 | April 29 | sky blue* | - | - |
| | May 1 | sky blue | yellow | yellow |
| | May 2 | sky blue | sl. sky blue | sl. sky blue |
| | May 3 | sky blue | sky blue | sky blue |
| | May 6 | sky blue | sky blue | sky blue |
| Phenol red (yellow-red) 6.8 - 8.4 | April 29 | reddish | - | - |
| | May 1 | brownish red | - | - |
| | May 2 | brownish red | yellow | yellow |
| | May 3 | brownish red | brownish red | yellow |
| | May 6 | red | red | light brown |
| | May 15 | red | red | red |
| Thymol blue (yellow - blue) 8.0 - 9.6 | May 6 | yellow | yellow | yellow |
| | May 15 | yellow | yellow | yellow |

* With brom thymol blue a green or sky blue color is formed instead of a deep blue. The suspension of solids in the silage juice was found to cause this difference in color.


GRAPH NO. 1.
TEMPERATURE
PH VALUE
YEAST
MOLD
PH VALUE
F.
140.
120.
100.
80.
60.
40.
9
8
7
6
5
4
3
15.
12.5
10.
7.5
5.
2.5
0
YEASTS AND MOLDS IN MILLIONS.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 17 19 21 23 25
NUMBER OF DAYS.


GRAPH. NO. 2.
TEMPERATURE
PH VALUE
YEASTS
MOLDS
PH VALUE.
F.
140.
120.
100.
80.
60.
40.
9
8
7
6
5
4
3
15.
12.5
10.
7.5
5.
2.5
0
YEASTS AND MOLDS IN MILLIONS.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 17 19 21 23 25
NUMBER OF DAYS.


GRAPH NO.3.
TEMPERATURE
PH VALUE
YEAST
MOLD
PH VALUE
F.
140.
120.
100.
80.
60.
40.
9
8
7
6
5
4
3
17.5
15.
12.5
10.
7.5
5.
2.5
0
YEASTS AND MOLDS IN MILLIONS.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 17 19 21 23 25
NUMBER OF DAYS.

The $P_H$ value for the initial and final determinations as determined with the use of the potentiometer were as follows:

| | Sample #1 | Sample #2 | Sample #3 |
|---|---|---|---|
| Normal silage | 3.719 | 3.060 | - |
| Abnormal silage | 7.371 | 8.030 | 7.709 |

Discussion of Part II

In general, the three samples of silage went through similar changes in the abnormal fermentation. These changes can be pictured as follows: During the first few days there is no appreciable change in the normal silage. However, during this period the yeasts have increased in numbers sufficiently so that they have become predominate. The next two or three days there is a marked increase in yeast development, the temperature increases and acid is destroyed. The temperature rises to about 120 degrees F. at which point the yeasts are apparently destroyed A slight reduction in heat through radiation occurs. The H ion concentration has decreased to between 6 and 7 by this time. This increase in temperature has made conditions favorable for the growth of molds that are present. As the mold develops in the yeasty silage a matted appearance is caused by the binding effect of the mycelium of the mold. The mold growth causes the temperature to rise to a higher level, it reaching 130 degrees F. or above. There has been destruction of the acids, first by the yeasts and then later by the molds. With the acidity partly reduced and finally destroyed entirely, the conditions are favorable for the development of alkaline producing types of bacteria which break down the protein and liberate ammonia. This results in further decrease in H ion concentration or alkalinity.

There was some difference in the abnormal fermentation of the silage in sample #3 as compared with samples #1 and #2. The factors in this difference appear to be a lower yeast content and higher acidity of the silage in Sample #3.

This caused a slower and more gradual increase in temperature, which made conditions favorable for mold growth. The molds developed and increased the temperature to a point where the yeasts were destroyed before as high a temperature as was usually produced by the yeasts was attained.

Part III of Experiment III

This part of the experiment involves the study of temperatures and yeast and mold counts, at the top of the silo.

Table VI gives the temperatures of the upper abnormal layer of silage, as found in large silos.

TABLE VI

Temperature changes in abnormal fermentation*

| Date | Dairy Silo #1 | Dairy Silo #2** | Dairy Silo #3 | Veterinary silo ** |
|---|---|---|---|---|
| | F. | F. | F. | F. |
| Aug. 26 | - | - | - | 110 |
| Aug. 29 | - | - | - | 110 |
| Sept. 3 | 118 | - | - | 106 |
| Sept. 4 | 132 | - | - | - |
| Sept. 6 | 128 | - | - | - |
| Sept. 8 | 124 | - | - | - |
| Sept.13 | 130 | - | - | - |
| Sept.16 | 126 | 120 | - | - |
| Sept.18 | 128 | - | - | - |
| Sept.23 | 136 | - | 135 | - |
| Sept.26 | 136 | 120 | - | 106 |
| Oct. 5 | 134 | - | - | - |
| Oct. 16 | 137 | 98 | - | - |
| Oct. 22 | 136 | 96 | - | 106 |
| Nov. 20 | 130 | 75 | - | 81 |

* Temperatures taken in large silos about 6" below the surface of the ensilaged material.

**Feeding from these two silos daily.

These temperatures are much higher than occur in the center of the silage mass and represent heat production in abnormal fermentation. It was found that there is a great variation in temperature, depending upon the depth at which it is taken. The distance from the wall was not found to be a factor except within limits of about one foot. At the bottom of the mold layer the temperature is the highest. Table VII gives the temperatures at different places and depths in two silos.

Table VII

Temperature at different locations in two large silos

| Distance from wall | Distance from surface | Dairy Silo #1 | Dairy Silo #3 |
|---|---|---|---|
| | | F. | F. |
| 1 foot | 12 inches* | 132 | - |
| 2 feet | 9 " * | - | 135 |
| | 19 " | - | 127 |
| | 26 " | - | 114 |
| | 30 " | - | 106 |
| 3 feet | 10 " | 134 | - |
| | 20 " | 124 | - |
| at center of silo | 5 " ** | 124 | - |
| | 9 " * | 136 | - |
| | 16 " | 124 | - |
| | 30 " | 114 | - |
| | 7 " * | - | 132 |
| | 17 " | - | 124 |
| 3 feet | 12 " * | 132 | - |
| 4 feet | 8 " * | - | 124 |
| 1 foot along side | 12 " * | 136<br>102 | |

* This distance down represents the distance to the apparently normal silage, just below the usual thick intertwined moldy mass.

** This is a couple inches above the thick intertwining mold and 4 inches above the normal silage.

A few more temperature observations were made . Silage was taken from two of the silos for feeding purposes soon after filling. Two piles of partly spoiled material were allowed to accumulate at the top of the silos. A portion of one of these that appeared yeasty had a temperature of 120 degrees F. and where mold predominated the temperature was 145 degrees F. In the other pile of spoiled material a temperature of 144 degrees F. was recorded. In the silo at the Veterinary Barn, twenty-four hours after re-filling a temperature of 94 degrees F. was recorded.

In silo #1 at the Dairy barn, the spoiled silage over an area of about four square feet was scraped off the top down to the normal silage. In a period of six days the normal silage molded down to a depth of six inches with an increase in temperature to 134 degrees F. The mold developed rapidly at this temperature and developed down to the depth where lack of air inhibited further growth. A definite line of demarkation was again formed between the normal and the abnormal silage.

In the silo at the Veterinary Barn, there was some mold down the wall with a temperature of 113 degrees F. Slight spoiling was evident around the edges to a depth of about 15 feet.

<u>The flora of the abnormal silage.</u>

Several platings were made for yeasts and molds but no plating was made for bacteria because counts of bacteria in former platings were variable due to a large number of spreaders, and it has already been demonstrated that a large number of spore forming alkaline producing types are present in silage of this kind. A description of the silage plated follows:

Sample #1 - Normal silage in process of heating.
Sample #2 - Moldy silage a few inches below the surface.
Sample #3 - Normal silage just below the thick layer of mold, where the silage is hottest.
Sample #4 - Silage that is slightly darkened in color with caramelized odor. This silage is about 9" from the surface and next to the thick compact mold growth, with temperature of 134 F.
Sample #5 - Normal silage one ~~inch~~ foot below #4.
Sample #6 - Normal silage inprocess of heating.

Table VIII gives the counts on these six samples.

TABLE VIII

Yeast and Mold counts of silage

| Sample Number | Date | Yeast Count | Mold Count | Reaction |
|---|---|---|---|---|
| | | Per gram | Per gram | $P_H$ |
| Dairy Silo | | | | |
| 1 | Sept. 6 | 379,000,000 | positive | 4 |
| 2 | Sept.13 | 244,000 | 4,600,000 | 7.2 |
| 3 | Sept.13 | 0 | 500,000 | 4.2 |
| 4 | Sept.24 | 0 | 21,000 | |
| 5 | Sept.24 | 0 | 39,000 | |
| Veterinary Silo | | | | |
| 6 | Sept. 6 | 66,000,000 | positive | 4 |

The above table indicates that continued high temperature destroys the yeast cells but the molds survive and will develop if air is present. Samples from different depths were plated. To determine how far below the surface the yeast cells are destroyed.

Table IX gives yeast and mold counts at different distances from the surface.

TABLE IX

Relation of yeast and mold growth to temperature

| | Distance from surface | | |
|---|---|---|---|
| | 6 inches | 16 inches | 30 inches |
| Temperature, degrees F. | 134 | 124 | 114 |
| Yeast counts incubated at 37°C. | 0 | 0 | 0 |
| Yeast counts incubated at 20°C. | 0 | 0 | 0 |
| Mold count incubated at 37°C. | 16,000 | 19,000 | 400,000 |
| Mold count incubated at 20°C. | 18,000 | 16,000 | 800,000 |
| $P_H$ value | 4.3 | 4.2 | 4.2 |

To substantiate further that the high temperatures in the upper few feet of silage destroy the yeasts, yeast cultures were made and inoculated on sterile silage in test tubes and buried in the silo at the above places. These tubes of silage inoculated with yeast were left buried for 7 days. They were then plated out and no yeast colonies developed.

This indicated that heat production in the upper layers of silage is a factor in silage preservation in so far as it destroys the yeasts.

It was noted that along the wall the silage did not get so hot and was abnormal. This suggested that yeasts might be a factor in spoiling next to the wall. A sample of silage at this point with a $P_H$ of about 6.4 and a temperature of 102 F. was plated. The yeast count was 1,300,000 and the mold count 600,000.

Discussion of Part III.

Both yeasts and molds are factors in the production of high temperatures in the abnormal fermentation of silage at the top of the silo. A temperature of 135 degrees F. is common in this abnormal fermentation. Yeasts appear to be the causative agents in producing the initial rise in temperature and will raise the temperature to 110-120 degrees F. and are then destroyed by the accumulated heat. Molds are responsible for a temperature of 135 degrees F. or higher. The maximum temperature recorded was 145 degrees F. The highest temperature was found at the junction between the thick layer of mold and the normal appearing silage immediately below it.

It seems as though the continued high temperature at the top of the silage is due to the mass being well insulated at the top and the small amount of heat that is given off at the top of the silo is compensated for by the heat produced by the mold growth.

The presence of air is the factor which determines whether or not abnormal fermentation will take place.

The plating of silage revealed high yeast counts in the initial stages of heating, and in abnormal silage at the surface, where the heat did not accumulate

due to radiation. The yeasts were destroyed to a depth of at least 30 inches, on account of the maintenance of high abnormal temperatures.

At the walls the spoiling goes down to varying depths, six or seven feet being quite common. If the silo walls leak air abnormal silage will result next to the walls. Some of the early investigators considered that heat was lost next to the wall which resulted in the improper fermentation of the silage. It would appear that two factors are concerned: The silage is not packed so closely next to the wall which allows air to permeate the outer portion and it may mold or decay. The yeasts develop in the presence of air and reduce the acidity with heat production. The heat is absorbed by the cool wall so that sufficient heat does not accumulate to destroy the yeasts. Therefore they continue to develop at the walls of the silo and continue the destruction of acid. This eventually allows the putrefying types of bacteria to break down the protein. It is different away from the walls because heat accumulates and destroys the yeasts. This high temperature is favorable to mold growth which results in a thick matting near the surface.

## Experiment IV

### The inoculation of corn silage and green corn with yeast and mold

The previous experiment demonstrated that in the presence of air silage or green fodder undergoes abnormal fermentation. It was shown that there is a complex flora, consisting of bacteria, yeasts and molds. This experiment aims to study the effects of the latter two factors, namely, yeasts and molds, separately and in combination when inoculated onto sterile silage and sterile green fodder.

Object of Experiment.

To study the changes in temperature and chemical reaction when silage and green fodder are inoculated with yeast alone, mold alone and a combination of yeast/mold and

Plan of experiment

Yeast and mold colonies were picked from platings of abnormal silage and cultured in boullion. It was aimed to get as many different species of yeasts and molds in the boullion cultures as could be picked from the plates. No attempt was made to identify them by a detailed study.

The silage and green fodder were sterilized in the autoclave for two hours at 15 pounds pressure.

Temperatures were recorded with an ordinary dairy thermometer and change in reaction was detected with hydrogen ion indicators.

Experimental data.

Sterile silage was inoculated with a culture of yeasts. Table X gives the rate of change in temperature.

TABLE X

Relation of yeast growth in silage to temperature

| Date | | Temperature of silage | Room Temperature |
|---|---|---|---|
| | | Degrees F. | Degrees F. |
| Feb. 13 | 10 a.m. | 64 | 74 |
| | 4 p.m. | 66 | 73 |
| Feb. 14 | 9 a.m. | 69 | 73 |
| | 4 p.m. | 70 | 73 |
| Feb. 15 | 9 a.m. | 83 | 67 |
| | 5 p.m. | 84 | 72 |
| Feb. 16 | 9 a.m. | 82 | 72 |
| | 5 p.m. | 81 | 76 |
| Feb. 17 | 9 a.m. | 81 | 74 |
| | 4 p.m. | 81 | 74 |
| Feb. 18 | 9 a.m. | 75 | 65 |

The normal silage gave a yellow color with brom cresol purple, while the abnormal silage gave a purple color indicating that the acidity had been reduced in the abnormal fermentation.

This was followed, by inoculating about 15 pounds of sterile silage in each case supposedly with yeasts alone, molds alone, and a combination of yeasts and molds. They were not in pure culture and the temperature readings did not represent the result of only these factors. The maximum temperature on yeast alone was 124 degrees and for yeast and mold in combination 128 degrees. Bacterial plates showed the presence of numerous bacteria. This silage was allowed to decay over a period of about five months and it changed from a normal silage to a black, watery, humus-like mass. The $P_H$ value changed from about 4 to about 8.3

The above experiment was repeated using about 10 pounds of normal sterile silage in each case To insure fairly pure cultures, the different types of yeasts and molds were cultured in bouillion and eplated. Table XI gives the temperature changes.

Table XI

Relation of yeast and mold growth in silage to temperature

| Date | Temperature of silage | | | Room Temperature |
|---|---|---|---|---|
| | Yeast alone F. | mold alone F. | yeast & mold F. | F. |
| Oct. 30 | 67 | 67 | 67 | 64 |
| 31 | 66 | 67 | 67 | 60 |
| Nov. 1 | 67 | 67 | 67 | 60 |
| 2 | 67 | 68 | 67 | 67 |
| 3 | 71 | 68 | 69 | 58 |
| 4 | 79 | 68 | 75 | 56 |
| 5 | 70 | 67 | 74 | 60 |
| 6 | 62 | 60 | 64 | 52 |
| 7 | 60 | 56 | 63 | 52 |
| 8 | 59 | 57 | 61 | 53 |
| 9 | 62 | 62 | 62 | 54 |
| 10 | 64 | 62 | 63 | 54 |
| 11 | 74 | 62 | 73 | 62 |
| 13 | 60 | 59 | 70 | 52 |
| 14 | 62 | 64 | 65 | 52 |
| 15 | 57 | 62 | 62 | 50 |

Table XII gives the temperature changes when sterile, green fodder is inoculated with cultures of yeasts, molds and yeasts and molds in combination. The green fodder had a moisture content of 60.57 percent, as determined on the air dry basis.

Table XII

n Relation of yeast and mold growth in green fodder to temperature

| Date | Temperature of Silage | | | Room Temperature |
|---|---|---|---|---|
| | yeast alone | mold alone | Yeast and mold | |
| Oct. 2 | 71 | 71 | 71 | 74 |
| " 3 | 77 | 77 | 81 | 65 |
| " 4 | 82 | 89 | 94 | 65 |
| " 5 | 91 | 92 | 99 | 74 |
| " 6 | 83 | 85 | 85 | 55 |
| " 7 | 70 | 76 | 74 | 55 |
| " 8 | 62 | 64 | 63 | 48 |
| " 9 | 60 | 61 | 64 | 51 |
| " 10 | 78 | 73 | 78 | 56* |
| " 11 | 78 | 77 | 78 | 48 |
| " 12 | 66 | 66 | 68 | 48 |
| " 13 | 58 | 58 | 62 | 48 |
| " 14 | 60 | 64 | 62 | 52 |
| " 15 | 68 | 69 | 68 | 57 |
| " 16 | 65 | 70 | 69 | 50 |
| " 17 | 64 | 69 | 67 | 54 |
| " 19 | 66 | 61 | 61 | 49 |
| " 20 | 73 | 64 | 74 | 49** |
| " 21 | 77 | 77 | 76 | 60 |
| " 22 | 83 | 83 | 84 | 59 |
| " 23 | 70 | 72 | 77 | 54 |
| " 24 | 66 | 67 | 70 | 52 |

* 300 c.c. sterile silage juice was added to each.

** 1/3 pound of sucrose in 333 cc water was added to each.

Discussion of results.

The temperature readings, as reported in Table XII, suggest that the juice of the green corn contains materials, as plant acids and sugars available for oxidation. This available material is soon used up and the temperature goes down. The addition of sterile silage juice resulted in an immediate rise in temperature with a later decline. The addition of sucrose also caused a rise in temperature. The addition of acid silage juice or sucrose caused an increased activity of the yeasts and molds due to the addition of available oxidizable material. This was responsible for the rise in temperature.

The acidity was promptly reduced by the yeasts and molds.

The sterile corn silage and green corn did not develop as high a temperature, when inoculated with pure cultures of yeasts, and molds alone and in combination, as did normal silage when it went through the process of abnormal silage fermentation. The factors which might explain this difference are, that smaller quantities of fodder or silage were used in the inoculation or that the cultures inoculated may not have included all the types of molds and yeasts that are present in abnormal silage, or symbioses with bacteria having a part might be responsible for this difference in temperature.

## Experiment V

Object of experiment. To determine some of the changes in chemical composition when normal silage undergoes abnormal fermentation.

Plan of experiment. The three normal and three abnormal samples of silage that were used in the study of the changes in flora, temperature and hydrogen ion concentration, were analyzed for ash, crude protein, ether extract, crude fibre, starch, total sugar, alcohol soluble total solids and non-protein. The juice of these six samples was titrated to determine acidity and alkalinity and the $P_H$ values were determined on the juice with a potentiometer. The moisture was determined on the original samples by air drying. All the samples were ground

up finely before analysis.

Methods of analysis.

The following determinations were made on the original materials;

The acidity and alkalinity were determined by titration using approximately n/10 sodium hydroxide or hydrochloric acid. The figure 80 was taken as the molecular weight for the basis of calculation. The $P_H$ values were determined with a potentiometer. The ash, crude protein and ether extract were determined according to the methods of the Association of Official Agricultural Chemists. The crude fibre was determined by the Kennedy modification of the method of the Association of Official Agricultural Chemists.

For the determination of total sugars, non -protein, starch and alcohol soluble total solids a separation of these compounds from each other is essential. The following extraction was applied for the above purpose: thirty grams of each of the six samples were extracted with 250 cc. of 80 percent alcohol, using large Soxhlet extractors, until the alcohol solution going over gave a negative test for sugar with alpha naphthol. The alcohol solution was condensed down in vaccuum at a low temperature to evaporate the alcohol, and then the remaining material was diluted up to 250 c.c. with distilled water. This 250 cc. represents the total alcohol soluble material present in 30 grams of silage.

The following determinations were made on the filtrate:

Total sugars by the picric acid method. Non-protein nitrogen by adding 50 cc. of 15 percent trichloracetic acid to 75 cc. of the filtrate to precipitate out any alcohol soluble true protein that might be present. They were filtered and Kjeldahl's were run on 50 cc. portions in duplicate to determine the nom-protein nitrogen. Alcohol soluble total solids by evaporating 25 cc. portions of filtrate to dryness. The nitrogen multiplied by 6.25 was taken as the non-protein.

The following determination was made on the residue;

Starch was determined, on the residue, according to the Association of Official Agricultural Chemists method combined with the picric acid method for the determination of sugar. The sugar content is multiplied by 0.9 to give the starch content.

The true protein was calculated by difference between crude protein and non-protein. The starch and sugar were calculated as starch by multiplying the sugar by 0.9 and adding the starch to the figure obtained.

Table XIII gives the results of the chemical analyses of normal and abnormal silage. All the determinations, except the $P_H$ values were run in duplicate and the figures reported represent the average of the two determinations.

Table XIII

The composition of three samples of silage before and after abnormal fermentation

| | Sample No. 1 | | Sample No. 2 | | Sample No. 3 | |
|---|---|---|---|---|---|---|
| | Normal | Abnormal | Normal | Abnormal | Normal | Abnormal |
| Acidity | 2.82 | - | 4.08 | - | - | - |
| Alkalinity | - | .504 | - | .824 | - | .512 |
| $P_H$ value | 3.719 | 7.371 | 3.060 | 8.030 | - | 7.709 |
| Ash | 7.18 | 9.75 | 6.38 | 10.34 | 6.77 | 10.12 |
| Crude protein | 6.71 | 12.03 | 5.84 | 9.60 | 6.22 | 9.62 |
| Non-protein | 3.07 | .67 | 2.76 | 1.16 | 2.92 | .54 |
| True protein | 3.64 | 11.36 | 3.08 | 8.44 | 3.30 | 9.08 |
| Ether extract | 4.02 | 10.50 | 4.37 | 11.57 | 4.58 | 6.13 |
| Crude fibre | 25.82 | 22.75 | 25.69 | 26.55 | 30.05 | 31.63 |
| Total sugar | 1.28 | 1.47 | 1.14 | 1.07 | .99 | .66 |
| Starch | 13.16 | 3.54 | 6.81 | .76 | 6.13 | .74 |
| Alcohol soluble total solids | 12.07 | 7.67 | 19.03 | 8.57 | 13.37 | 6.97 |
| Starch and sugar calc. as starch | 14.312 | 4.863 | 7.836 | 1.723 | 7.021 | 1.334 |

The results as reported in Table XIII, demonstrate that chemical changes take place when normal silage undergoes abnormal fermentation. The figures present the actual percentages of the constituents found in the different samples. Hence, the percentages, as reported, are relative rather than absolute. The absolute percentages are determined from the relative percentages by calculation using the following formula:

$$\frac{Ash_N \times X_A \times 100}{Ash_A \times X_N} = \text{Absolute percentage}$$

N = normal silage

A = abnormal silage

X = Any one of the constituents.

It is assumed that there has been no change in ash content.

The results of this calculation are reported in Table XIV.

Table XIV

The composition of abnormal silage, based on ash taken as 100 percent.

| | Sample No. 1 | Sample No. 2 | Sample No. 3 |
|---|---|---|---|
| | % | % | % |
| Ash | 100.00 | 100.00 | 100.00 |
| Crude protein | 132.03 | 101.43 | 103.41 |
| Non-protein | 16.07 | 25.94 | 12.38 |
| True protein | 229.83 | 169.10 | 184.07 |
| Ether extract | 192.34 | 163.37 | 89.54 |
| Crude fibre | 64.89 | 63.77 | 70.42 |
| Total sugar | 84.57 | 57.92 | 44.60 |
| Starch | 19.88 | 6.89 | 8.08 |
| Sugar and starch Calc. as starch | 25.04 | 13.57 | 12.71 |
| Alcohol soluble total solids | 46.78 | 27.78 | 34.88 |

Discussion of results.

The chemical analyses of the three samples of silage reveals that the ash increases about 50 percent. This indicates that there is a loss of about 33 percent in organic matter. This loss in organic matter falls largely upon the acid, crude fibre, sugar and starch.

The abnormal fermentation of normal silage results in the total destruction of acidity. The reaction is changed from acid to alkaline as is indicated by the change in $P_H$ value from 3-4 to 7.3 - 8.0.

The nitrogen compounds undergo marked changes. There is a slight increase in crude protein, which might be explained by nitrifying types of bacteria assimilating free nitrogen of the air and converting it into protein. The non-protein undergoes a marked decrease in amount which can be explained by micro-organisms, possible molds being of greatest importance, building up the simpler nitrogenous compounds, as amino acids, into true protein. There is a marked increase in true protein.

The ether extract increased in two and decreased in one of the samples.

The alcohol soluble total solids decreased in all three samples.

The crude fibre decreased about one-third in total amount in all three samples.

There is a marked decrease in starch and sugar. It appears as though the micro-organisms break down the starch into sugar, and the sugar is oxidized with the production of heat and liberation of carbon dioxide and water. The breaking down of starch into sugar and the further breaking down of sugar appear to go along somewhat parallel, with a supply of sugar always present. In samples number two and three, the starch reserve and available sugar were lower than in sample number one.

## CONCLUSIONS

1. Corn silage has sufficient acid normally, to inhibit the development of putrefying types of bacteria.
2. Corn silage with a moisture content of 70 percent will in most cases be free from mold.
3. In the abnormal fermentation of silage, yeasts, and molds have an important part in acid destruction and heat production.
4. The putrefaction of silage is due to the development of alkaline producing types of bacteria.
5. In the abnormal fermentation of silage there is a marked initial increase in the numbers of yeasts followed by a rapid decrease, with an increase of molds and bacteria.
6. The changes occurring in the abnormal portion of silage, at the top of large silos, are similar to those which take place when normal silage goes through the process of abnormal fermentation.
7. The maximum temperature found in the top layers of silage in large silos, was 136 degrees F. This high temperature occurs about nine inches below the surface at the junction between the moldy mass and the normal silage immediately below. Fromthis point was found a gradual decline to 114 degrees F., thirty inches below the surface.
8. The maintenance of high abnormal temperatures in the upper layers of normal silage destroys the yeasts.
9. The chemical changes resulting when normal corn silage undergoes abnormal fermentation are a total destruction of acidity and a change in $P_H$ from 3.0-4.0 to 7.3-8.0. The results indicate upon analyses of three samples, that there is a loss of about 33 percent in organic matter. This loss involves a total destruction of acidity, a decrease of about one-third in crude fibre, a decrease in sugar and a very marked decrease in starch. There was a slight increase in crude protein, a marked decrease in non -protein and a marked increase in true protein. There was also a decrease in alcohol soluble total solids.

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