Historical Document
Kansas Agricultural Experiment Station

JANUARY, 1916. TECHNICAL BULLETIN No. 2.

AGRICULTURAL EXPERIMENT STATION.

KANSAS STATE AGRICULTURAL COLLEGE.

SOME IMPORTANT FERMENTATIONS IN SILAGE.

MANHATTAN, KANSAS.

KANSAS STATE PRINTING PLANT.
W. R. SMITH, *State* Printer.
TOPEKA. 1916
6-2061

Historical Document
Kansas Agricultural Experiment Station

SUMMARY.

1. The production of silage is the result of fermentation processes.
2. The major part of this fermentation is due to the activities of microorganisms.
3. The microorganisms consist of four prominent groups: (1) The acid group; (2) the colon group; (3) yeasts; and (4) a miscellaneous type.
4. The most important fermentation thus far noted is that of acid production.
5. A group of organisms which will classify as belonging to the Bulgarian group is responsible for the high acid content of normal and good silage.
6. It is suggested that the greater part of acetic acid production is the result of the Bulgarian and colon fermentation.

Historical Document
Kansas Agricultural Experiment Station

Some Important Fermentations in Silage.

O. W. HUNTER and L. D. BUSHNELL.

A MORE definite knowledge of the ripening changes occurring in silage is of fundamental agricultural importance. The aim in the present investigation is to make a detailed study of the fermentation processes common to good silage. The results presented in this bulletin constitute a report of progress, rather than the final results, of an extensive investigation now under way at this station. They may explain however some of the important changes which take place in nearly all normal silage fermentation.

WORK OF PREVIOUS INVESTIGATORS.

Literature involving a biological study of silage is somewhat limited. Babcock and Russell (1) have demonstrated that good silage can be made under laboratory conditions when the bacterial activity is checked by saturating the siloed material with antiseptics. They believe the initial heating to be accomplished by the respiratory processes of the cut plant tissue, the activity to be the result of intramolecular respiration, and the putrefactive changes to be due to bacteria.

Likewise E. J. Russell (2) states that the characteristic silage changes are the disappearance of sugar, the less resistant celluloses, and part of the proteins, with the formation of fatty acids, hydroxy acids, amino acids, diabasic diamino acids, purin bodies, and other bases. The agents concerned were assumed to be the living maize cell, the enzymes and the microorganisms, the first two being the cause of the primary and essential changes, and the last being only of a secondary nature. The formation of acetic and butyric acid seems to be a respiration effect and comes about when the living cells are deprived of oxygen.

Franco Samarani (3) after many years of experimenting to discover the best method of preparing silage, states that in ordinary silos the grass, after the first few days, undergoes two typical and different fermentations at the expense of the sugar and cell sap—acetic and lactic acid fermentation. He

Historical Document
Kansas Agricultural Experiment Station

believes the first to be due to an intramolecular process through which the sugars are transformed into alcohol and carbonic acid, as a result of the vital activities of the cell in the relatively warm medium of the silo, and in the almost complete absence of oxygen the alcohol is converted into acetic acid, due probably to a chemical or physiological, but certainly not a bacteriological, action. The second process is an ordinary lactic acid fermentation due to the action of the bacilli and cocci common in milk fermentation. The acid content was found on the average to be 70 percent acetic, 10 percent butyric and 20 percent lactic. The large percentage of acetic acid was assumed to be the result of the high temperature, 40° C. to 60° C., which favored intramolecular fermentation. He noted that fermentation was usually complete in ten days and suggested that the undesirable fermentation could best be prevented by the addition of a dilute solution of milk sugar to the silage. By the use of a mechanical press the lactic acid fermentation was favored on account of the pressure driving out the oxygen, thus lowering the temperature and making it favorable for the lactic acid bacteria. When this press was used the analysis showed 70 percent lactic and 20 percent acetic acid, practically the reverse of the former condition.

C. Gorini (4) believes the addition of lactic acid organisms to fermenting silage will greatly help the resultant product. After ten years of investigation he distinguishes four types of silage—that with butyric acid bacteria predominating; that with lactic acid bacteria predominating; that with putrefactive bacteria predominating, and that free from bacteria. The first two classes of silage he states are normal, while the last two are abnormal. Heat is essential to successful silage fermentation, but too high a temperature destroys, while too low a temperature does not favor proper development. The optimum temperature, 50° C. (122° F.), favors lactic acid bacteria, while at 60° C. butyric acid organisms predominate.

Esten and Mason (5) differ from most of the previously named investigators in that they believe the important fermentation changes in silage are due to microörganisms and not to the enzymes of tissue cells. They conclude:

1. The fermentation of corn silage is essentially the change of sugar into several acids, the most important being the change by lactic acid bacteria into lactic acid. A secondary change is produced by the action

Historical Document
Kansas Agricultural Experiment Station

of yeasts on the remaining sugar, changing it into alcohol, while the acetic acid bacteria change it into acetic acid.

2. The acid bacteria are divided into three divisions:
   a. Those that ferment the three sugars, dextrose, lactose and saccharose, and that curdle milk.
   b. Those that ferment lactose rather feebly, but produce more acid in silage than those included in the first division.
   c. Those that do not ferment lactose.

The first two groups were considered to be the ordinary milk-souring bacteria, while the third is a new species.

3. Exclusion of air is necessary for the proper production and preservation of silage.

4. Silage undergoes a ripening somewhat similar to cheese, which softens the fibers, makes the proteins more digestible, and adds new and agreeable odors. This ripening process occupies three to four weeks.

The relation of acids to silage fermentation has been studied by Dox and Neidig (6, 7), who conclude as follows:

1. Lactic acid is normally present in silage in excess of the volatile acids. The average ratio is 1.0 to 0.75.

2. The form in which lactic acid occurs in silage is the optically inactive or racemic mixture.

3. Volatile aliphatic acids occur in considerable amounts in all of the samples of good silage examined.

4. Acetic acid comprised about nine-tenths of the total volatile acids present, propionic being next in importance.

5. Butyric acid was found only when there were some conditions of spoiling which were otherwise noticeable.

6. Alcohols were found in appreciable quantities, but only traces of esters.

RIPENING PROCESS CONCERNED IN SILAGE.

The ripening changes occurring in silage are the result of fermentation. The word fermentation is here used as referring to all chemical changes produced by enzymes, either directly or indirectly. The chemical changes brought about directly by enzymes are due to the action of the enzymes of the plant cells or microörganisms upon the cut forage plant. Those chemical changes brought about indirectly by enzymes are due to their by-products acting upon the plant tissue. The formation of silage, therefore, is due to two chief processes, each, no doubt, dependent upon the other:

1. The biological action resulting from either the enzyme action of microörganisms or of tissue cells.

2. The chemical action due to the by-products of direct enzyme action, acting upon the siloed material.

Historical Document
Kansas Agricultural Experiment Station

FACTORS NECESSARY FOR PROPER FERMENTATION.

Several important factors are necessary for any forage plant to ferment into normal silage. It is the object in this paper, however, to discuss only those factors which are important from a fermentation standpoint. No definite statement can be made concerning the relative importance of these factors. All are necessary for proper fermentation and dependent upon each other to a large extent.

Proper material for silage. The first prerequisite for good silage is proper forage. The material usually employed for this purpose is limited, due largely to the lack of experience with other plants. There is no doubt that, with a little experience, several other crops could be demonstrated practicable for this purpose. At the same time it must be admitted that the best silage crops are probably those now in general use. In choosing suitable material for the preparation of silage two factors should be considered — its ability to undergo the proper fermentation, and its practical value from the feeding standpoint. The chemical composition of the substances will influence both greatly, especially the fermentation. The carbohydrate content of the forage is most essential, inasmuch as the predominating fermentative changes occurring in the silage result from the decomposition of these compounds.

Proper moisture content. Although moisture is a most essential factor in the production of silage, it is a factor that does not need especial attention in the siloing of the common freshly cut crops. The moisture content of the plant at this time is sufficient. However, when certain fodder crops are siloed, the moisture content must be increased to replace that lost by evaporation. One of the important requirements for the growth of microörganisms is a proper degree of moisture. The exact amount necessary for active growth can not be stated, as the concentration and solubility of the substance must be considered. In silage, however, as in most cases, a moisture content of 70 percent gives a very favorable condition for active fermentation, while a moisture content of less than 25 percent is too low and will give abnormal conditions for fermentation.

Anaerobic conditions. Good silage, even with the right forage plant, is dependent on proper fermentation. The desira-

Historical Document
Kansas Agricultural Experiment Station

ble microorganisms for this purpose develop best, while in the silo, when air is excluded, hence the nearer true anaërobic conditions are approached in the silo, the better the silage quality will be. Furthermore the most destructive of the undesirable microorganisms require aërobic conditions, and their development is consequently inhibited in the absence of air.

Proper enzymes. That enzymes are the most essential factors in silage formation can not be denied. It is most important, then, if silage is the product of fermentation, to have the proper agents present to initiate these processes. In practice, no direct attention has been given this subject in relation to the siloed material, although they are the chief agents of fermentation. If the enzymes from the cut plant tissue alone are responsible for this fermentation they will be present in sufficient quantities in or on the material as it enters the silo to produce the proper changes. Again, if the enzymes are of microbial origin there will usually be found sufficient numbers of these microörganisms on the cut forage, as it enters the silo to provide a source for such enzyme activity. In either case the progress of the proper fermentation will depend upon other factors necessary for fermentation. The enzymes of microörganisms are without doubt the chief agents in silage ripening. As a more thorough understanding of silage fermentation is obtained it may be practicable to use pure cultures or starters to produce a better flavored and more uniform silage. This has been tried successfully by C. Gorini (4).

Proper temperature. The activities of microorganisms are influenced largely by temperature conditions. The optimum temperature for the majority of organisms lies between 25° C. and 40° C. The temperature produced from the fermenting siloed plants is sufficient to give optimum temperature conditions for the majority of organisms concerned. It is a noteworthy fact that while the increase in temperature is a result of fermentation, it is most essential for the completion of the ripening process.

THE RESULTS OF FERMENTATION.

In studying the ripening changes in silage three important stages can be noted, which appear to follow one another in a more or less logical order, namely: (1) A rise in temperature,

Historical Document
Kansas Agricultural Experiment Station

(2) an increased acidity followed very closely by the (3) production of odors.

One of the first changes observed in any freshly siloed material is the production of heat. The degree of temperature and rate of increase will vary. The factors affecting this are: the amount of air within the silo, governed by the compactness of the siloed material and the hermetic condition of the silo itself; the heat radiation due to the size of silo; and the season. On this account no definite temperature can be given for a normal fermenting silage. From the silos studied it has been observed that the temperatures all show a gradual rise until the maximum is reached, and finally a steady decrease. The maximum temperatures recorded for the silos studied were 29° C. to 46° C., while the average temperature was 37.5° C. This temperature refers to the center of the silo.

The increase in temperature is usually prompt and rapid, the maximum being reached within one or two weeks under normal conditions. However, if the silo is filled in the middle of winter, as it frequently is, the rise in temperature is much slower and the maximum degree will be reached much later.

With the rise of temperature there is also an increased acidity. The rate of acid formation is rapid after the true desirable fermentation starts. The maximum acidity is normally reached within fourteen days, often before. The production of odors follows closely the acid production, so that by the time the silage has reached its maximum acidity, true silage odor is also present.

The major fermentations concerned in the ripening of silage are normally complete within two weeks.

PROGRESS OF THE PRESENT INVESTIGATION.

The primary object in carrying on these investigations is to obtain more specific information concerning the fermentation of various kinds of silage. A very large percentage of the work accomplished to date has been of a preliminary nature. Much time has been spent in determining the microbial flora of different kinds of silage and in making a quantitative and qualitative study of the same.

Over three hundred examinations were made from different kinds of silage at various stages of fermentation. The silage used was that prepared in the laboratory and from ordinary silos.

Historical Document
Kansas Agricultural Experiment Station

The silage made under laboratory conditions was prepared by cutting the material in small pieces and packing tightly in quart milk bottles. The bottles were sealed air-tight by the use of rubber stoppers and wired in. The silage thus prepared resembled the silage made by the usual method, in respect to the bacteriological and chemical analyses, together with the odors. After numerous examinations it is the authors' opinion that the silage prepared under laboratory conditions gives results comparable to those obtained from actual silos and offers a practical and accurate means of studying the fermentation of silage.

METHODS OF PROCEDURE.

To obtain the silage for analysis from the silos, a small hole about two inches in diameter was bored in the side of the silo about two or three feet from the level of the ground. If the silo was cement, the holes were made in the doors. From these holes samples of silage were collected in sterile containers with a large extension auger. After the required amount was obtained the hole was tightly stoppered with paraffined corks. The general practice was to collect the sample from a new hole each time.

One objection to this, method is that it allows air to pass into the fermenting forage. However, it is believed that the air thus admitted plays a very small part in checking the proper fermentation. The space left is soon filled by the pressure from above, and the air admitted will not circulate very far before being consumed.

BACTERIOLOGICAL TECHNIQUE.

Twenty grams of the silage were placed in 200 cc. of a sterile physiological salt solution and thoroughly shaken. This was further diluted by the use of similar water blanks, for obtaining dilutions for the plates.

Plain agar was used for determining the total numbers of the microörganisms.

Plain gelatin was used to determine the protein digesting types. Not having a satisfactory place for the incubation of gelatin plates, sterile tubes of gelatin were inoculated with varying dilutions of silage, and incubated at 35° C. for five days.

Historical Document
Kansas Agricultural Experiment Station

The number of liquefiers was determined by placing the gelatin tubes in the ice box, after removing them from the incubator. From previous experience it has been found that a tube of digested gelatin will not solidify on cooling. Hence by noting the highest dilution showing liquefaction after cooling, the number of liquefiers can be estimated. By the use of small dilutions and more tubes, the results are probably more accurate than those obtained from the usual method of counting the liquefying colonies on gelatin plates.

Glucose acetic acid agar was used to determine the total number of the Bulgarian types of organisms present. The composition was one percent glucose agar, to which was added one cc. of a one percent sterile acetic acid solution. The acid solution was added directly to the plates and the glucose agar was mixed with it when the plates were poured. Lactose agar was used in place of glucose agar in the preliminary work, but from subseqent work it was observed that the glucose medium favored this type of organisms more than the lactose agar. The small amount of acid added was sufficient to check practically all types of organisms except the Bulgarian group and the yeasts. After a little experience there was no difficulty in differentiating between these two types, on account of their characteristic colonies.

Glucose litmus broth was used to determine the total number of acid producers. It was prepared by using one percent glucose broth to which a few drops of litmus solution had been added. Several tubes of this medium were inoculated with different dilutions of the silage infusion and incubated. The total acid producers were determined by noting the acid reaction in the highest dilution present.

This method was used in place of a litmus agar medium because a more accurate estimate of the acid producers could be thus obtained. On a litmus agar plate, if the acid colonies are few and other types predominate, the colonies may fail to appear acid on account of the acid being neutralized by the alkaline by-products from the other types present. The organisms in the glucose broth solution are not held in one place, as they are in the solid agar media. This, together with the fact that the glucose broth acts as an enrichment medium for the acid group, gives better opportunity for the acid bac-

Historical Document
Kansas Agricultural Experiment Station

teria to increase more rapidly than the miscellaneous organisms. Likewise, the acid produced from the more rapid-growing acid bacteria is sufficient to check the slower development of the miscellaneous type. The dilution method is more tedious, but providing the differences between the dilutions are small, the results obtained are more satisfactory and more accurate.

Bile lactose fermentation tubes were employed in determining colon fermentation. The Dunham fermentation tubes were inoculated with different dilutions of the silage extract. The tube with the highest dilution showing gas production was used as an estimate of the total number of colon organisms present. To further substantiate this presumptive test, different dilutions were plated out from time to time and the colon organisms isolated and identified.

Glucose fermentation tubes were used in determining the total number of yeasts present, by noting the gas production in the different dilution tubes. To be certain that the gas was due to yeast fermentation, stained preparations were made from each dilution and examined for the presence of yeast cells. If gas was present and no yeast could be demonstrated, it was taken for granted that the gas formation was not due to yeasts, but to other causes. The yeast count from the acid agar was used to check this method, and they compared very favorably. The general rule, however, was that a higher count was obtained from the fermentation tubes than from the plates.

All media used were made from Leibig's Beef Extract, and a reaction of + 1.0 to phenolphthalein required. The period of incubation, unless otherwise stated, was always four days, except with gelatin, which was for five days, at 35° C. The long period of incubation was used to favor the complete development of the Bulgarian type. The enumeration of all plates was done by the aid of a hand lens.

The principal and predominating types of microörganisms as they appeared on the different media from time to time were isolated. A morphological, cultural and biochemical study was made from the tubes thus isolated.

Stained preparations were made directly from the silage infusion in order to check the results from the cultural analyses. It seemed unnecessary to employ the use of synthetic media at

Historical Document
Kansas Agricultural Experiment Station

this time after comparing the microscopic appearances of the silage with the results obtained from the culture media, as the media was giving a very good estimate of the true microbial content of the silage.

CHEMICAL ANALYSES.

To obtain a more definite idea of the fermentation processes going on in the different kinds of silage, the degree of total acidity and the ratio of volatile to nonvolatile acids was determined in certain kinds of silage. The total acidity was determined by extracting the acid from 100 gms. of finely ground silage, by placing in 1000 cc. of distilled water, and shaking for one hour in a shaking machine. An aliquot portion of the filtered extract was used for determining the total acidity and volatile acids. A correction for the moisture content of the silage was made for each analysis.

The volatile acids were determined by the methods used by Dox and Neidig (6), distilling by steam under diminished pressure at a temperature of 80°-90° C. The moisture content was determined by the evaporation of the finely ground silage at a temperature of 110° C. for four hours, and weighing. The temperature of the silage was recorded by the use of a Leeds-Northrup temperature indicator and resistant bulbs. The resistant bulbs were buried in the silage, and connected with the surface with lead insulated wires.

ANALYSES OF VARIOUS KINDS OF SILAGE.

On account of the similarity of the data obtained from the different kinds of silage, only results of a few of the examinations made from each kind of silage will be given.

Kafir Silage. On September 25, 1914, kafir was placed in a cement silo having a capacity of about ninety tons. The kafir was in good condition and the heads were not removed. Samples were taken for analysis at frequent intervals until the major fermentation processes were complete, and at longer intervals after this until the silo was opened. The only chemical analysis made was obtained from the material as it went into the silo and on the last date examined.

The summarized results are found in table I.

Historical Document
Kansas Agricultural Experiment Station

TABLE I.

Date Sampled.	Days.	Bacteriological examination.						Temperature.	
		Quantitative estimation per cc.						Outside of silo.	Center of silo.
		Plain agar.	Liquefiers in gelatin.	Total acid producers.	Bulgarian group.	Yeasts.	Colon group.		
9–25–'14	0	30,000,000	1,000,000	100,000,000	.....	58,000	1,000,000	.....	.....
9–28–'14	3	400,000,000	500,000	100,000,000	40,000,000	37,000	1,000	24.5° C.	24.0° C.
9–30–'14	5	150,000,000	1,000	100,000,000	2,000,000	80,000	1,000	24.0° C.	25.5° C.
10– 2–'14	7	325,000,000	10,000	100,000,000	137,000,000	100,000	.....	23.0° C.	26.5° C.
10– 6–'14	11	8,000,000	10,000	1,000,000	5,000,000	100,000	50,000	22.5° C.	28.0° C.
10– 9–'14	14	15,000,000	10,000	50,000,000	10,000,000	130,000	50,000	21.0° C.	28.5° C.
10–13–'14	18	7,000,000	10,000	1,000,000	1,000,000	120,000	50,000	7.5° C.	29.0° C.
10–16–'14	21	3,000,000	50,000	1,000,000	600,000	200,000	500,000	13.5° C.	29.0° C.
10–23–'14	28	1,000,000	500,000	1,000,000	700,000	.....	10,000	20.5° C.	29.0° C.
10–30–'14	35	2,000,000	1,000	100,000	100,000	.....	.....	16.5° C.	28.5° C.
11–13–'14	49	115,000	5,000	50,000	100,000	.....	.....	10.0° C.	27.5° C.
11–25–'14	61	120,000	100,000	100,000	90,000	6,000	.....	11.0° C.	27.0° C.

Historical Document
Kansas Agricultural Experiment Station

The table shows both the quantitative and qualitative microbial content of silage. The following facts can be noted:

(a) A relatively high microbial content existed in the kafir as it was siloed. All types were represented except the Bulgarian group, which were present in such small numbers that they could not be noted.

(b) A rapid increase in the total number of microörganisms the first few days was followed by a decrease after the first week.

(c) A marked increase took place in the Bulgarian group for the first eight days, but a gradual decrease followed.

(d) The gelatin liquefiers proved to be unimportant. Their number decreased very rapidly after the material was siloed.

(e) A comparison of the total number of microörganisms with the total number of acid producers indicates the acid producers to be the predominating organisms.

(f) A gradual increase occurred at first in the number of yeasts, but they disappeared after about three weeks.

(g) A decrease was observed in the colon group during the first few days, followed by an increase and then final disappearance.

(h) An increase in temperature for the first eighteen days was followed by a gradual decrease.

This silage was considered very good. The acidity as it entered the silo was 0.18 percent figured as lactic acid. The final acidity was found to be 2.07 percent, of which 1.36 percent was nonvolatile, calculated as lactic acid, while 0.71 percent was volatile, figured as acetic acid.

Cane fodder silage. A small stave silo with a capacity of about ten tons, built for experimental purposes, was filled with cane fodder on March 15, 1915. The fodder had been shocked and stacked all winter and was in good condition. Enough water was added to the cut fodder as it went into the silo to bring up the required moisture content.

The data obtained are summarized in table II.

The conclusions derived from the above data are very similar to those obtained from kafir silage. Summarized, the data show:

(a) First, an increase and then a rapid to gradual decrease of all microörganisms.

Historical Document
Kansas Agricultural Experiment Station

TABLE II.

Date Sampled.	Days.	Bacteriological examination. Quantitative estimation per cc.						Chemical examination. Percent moisture.	Chemical examination. No. cc. N/10NaOH to neutralize acid in 100 gms. silage.			Temperature in centigrade.	
		Plain agar.	Liquefiers in gelatin.	Total acid producers.	Bulgarian group.	Colon group.	Yeasts.		Total acidity.	Total volatile acidity.	Total non-volatile acidity.	Surface temperature.	Center of silo.
3-20-'15.....	0	600,000,000	100,000	10,000,000	75,000	1,000,000	1,000,000	70	22.8	.....	.....	.....	.....
3-22-'15.....	2	750,000,000	10,000,000	100,000,000	85,000	10,000	70,000	65	28.2	.....	.....	1.5°	1.5°
3-25-'15.....	5	50,000,000	.....	1,000,000	7,000	1,000,000	100,000	60	44.8	.....	.....	27.5°	17.5°
3-29-'15.....	9	60,000,000,000	100,000,000	1,000,000,000	3,000,000	.....	500,000	70	117.7	.....	.....	44.0°	39.0°
4- 6-'15.....	13	60,000,000	10,000,000	10,000,000,000	10,000,000	100,000	1,000,000	40	291.2	135.2	156.0	45.5°	46.0°
4- 9-'15.....	20	25,000,000	10,000,000	100,000,000,000	12,000,000	.....	1,000,000	60	212.0	95.4	116.6	41.0°	40.0°
4-24-'15.....	35	10,000,000	1,000,000	1,000,000	5,000,000	.....	2,000,000	85	249.5	108.5	141.0	39.5°	35.0°
5- 6-'15.....	47	1,000,000	10,000	100,000	3,000,000	.....	100,000	70	288.9	171.2	117.7	.....	.....

Historical Document
Kansas Agricultural Experiment Station

(b) The presence of four groups of microorganisms, namely: the acid group in which the Bulgarian type predominates, yeasts, the colon group, and the gelatin liquefiers.

(c) The first three groups mentioned above are the important organisms, while the fourth group is of no importance in good silage.

(d) The increase in temperature, the acidity and the microbial content are nearly parallel.

The increase in temperature, acidity, and all of the important types of fermentation, were very slow as compared with normal silage fermentation. This can be explained by the fact that when the fodder went into the silo the outside temperature was freezing. The water, which was added to increase the moisture content, froze as it was added to the fodder. When the temperature began to rise, however, the fermentation progressed very rapidly.

There were comparatively large numbers of the Bulgarian group on the fodder as it was placed in the silo. This is accounted for by the fact that the fodder was stacked all winter, and during some of the warmer days of fall or winter proper conditions were produced which were conducive to their growth.

The silo was opened about the first of May, 1915. The silage was in normal condition and a good grade.

Alfalfa silage. This department, coöperating with the dairy and chemical departments, has been investigating the fermentation processes in silage made from alfalfa. As this work has not been completed, a full report can not be given. However, as good alfalfa silage has been made under laboratory conditions and fair silage made in silos, the results from one of the silos filled with alfalfa will be presented to show the similarity of the microbial flora to those of cane and kafir silage.

A small experimental stave silo, with a capacity of about ten tons, was filled with freshly cut alfalfa and rye in the proportions of two parts alfalfa to one part rye. The feed was cut into small pieces by a silage cutter as itwent into the silo.

Table III shows the results obtained from the examination:

Historical Document
Kansas Agricultural Experiment Station

TABLE III.

Date Sampled.	Days.	Bacteriological examination. Quantitative estimation per cc.							Temperature.	
		Total on plain agar.	Total on litmus lactose agar.	Total acid producers on litmus lactose agar.	Neutral type on litmus lactose agar.	Bulgarian group.	Yeasts.	Colon group.	Outside of silo.	Center of silo.
5-28-'14	0	15,000,000,000	10,000,000,000	3,000,000,000	7,000,000,000	13,000	10,000,000,000	1,000,000,000	24.0° C.	.....
5-30-'14	2	1,000,000,000	3,000,000,000	2,000,000,000	1,000,000,000	20,000,000	10,000,000	10,000,000	33.5° C.	38.0° C.
6- 2-'14	5	85,000,000	50,000,000	5,000,000	45,000,000	3,000,000	10,000,000	10,000,000	25.0° C.	38.0° C.
6- 5-'14	8	50,000,000	20,000,000	2,000,000	18,000,000	2,000,000	100	100	29.5° C.	37.0° C.
6- 8-'14	11	230,000,000	80,000,000	40,000,000	40,000,000	2,000,000	10,000	10,000	30.5° C.	35.5° C.
6-11-'14	14	1,000,000,000	1,000,000,000	500,000,000	500,000,000	6,000,000	10,000	10,000	32.5° C.	34.5° C.
6-15-'14	18	36,000,000	30,000,000	.....	.....	11,000,000	.....	.....	23.0° C.	34.0° C.
6-19-'14	22	3,000,000	350,000,000	50,000,000	300,000,000	8,000,000	100	10	33.5° C.	33.0° C.
6-26-'14	29	10,000,000	10,000,000	.....	.....	600,000	.....	.....	33.0° C.	31.0° C.
7- 3-'14	36	3,000,000	3,000,000	200,000	3,000,000	16,000	100	100	23.0° C.	31.0° C.
7-17-'14	50	5,000,000	5,000,000	2,000,000	3,000,000	3,000	1,000	10	21.5° C.	30.0° C.
10-30-'14	156	10,000,000	5,000,000	3,000,000	2,000,000	50,000	.....	.....	.....	23.0° C.

Historical Document
Kansas Agricultural Experiment Station

The acid producers were determined in this experiment by the use of litmus lactose agar instead of litmus glucose broth. The results obtained from the entire study are comparable with those mentioned in the previous tables.

The silage was of fair grade although the odor was not as pleasant as that of cane or corn silage. The material, however, was in good condition and was eaten readily by the stock. The total acidity at the end of about twenty-five weeks was 2.8 percent calculated as lactic acid.

EFFECT OF ANTISEPTICS ON SILAGE FERMENTATION.

Several investigators previously have stated that the major process in silage ripening is due to the action of the enzymes from the living tissue cells, while microörganisms play only a secondary part. To substantiate these theories, cane and alfalfa were siloed in milk bottles with and without the addition of antiseptics and the resulting fermentations studied.

Alfalfa silage. Two different experiments were made at different times. As a large amount of work had been done on alfalfa silage fermentation, alfalfa was used first, at two different stages of maturity, These two stages were (1) alfalfa which was fully matured and cured, and (2) alfalfa which was green and in about the first bloom stage.

A small amount of water was added to the cured alfalfa. Two percent by weight of dextrose sugar was added and mixed with each kind of alfalfa. The bottles which were treated with antiseptics received, about six percent of chloroform calculated on the basis of the total weight of alfalfa.

The alfalfa thus prepared was placed at room temperature and examined at frequent intervals to note the progress of fermentation. A new bottle was opened at each analysis.

Tables IV, V, VI and VII show the results obtained from the alfalfa thus treated.

The following brief conclusions can be summarized from tables IV, V, VI and VII.

(a) The microbial flora content of the untreated alfalfa was very similar to that of normal cane and corn silage.

(b) The microbial flora of the treated alfalfa shows a marked effectof the antiseptics; growth of the principal types of microörganisms being checked.

Historical Document
Kansas Agricultural Experiment Station

TABLE IV. Cured alfalfa without chloroform.

Date Sampled.	Days.	Bacteriological examination. Quantitative estimation per cc.					
		Plain agar.	Liquefiers in gelatin.	Total acid producers.	Bulgarian group.	Yeasts.	Colon group.
11-13-'14.....	0	10,000,000	.....	1,000,000	.....	100	10,000
11-19-'14.....	6	600,000,000	1,000	10,000,000	4,000,000	.....	100
11-25-'14.....	12	1,000,000,000	10,000	100,000,000	360,000,000	.....	.....
12- 8-'14.....	25	100,000,000	10,000	1,000,000,000	10,000,000	.....	.....
1- 9-'15.....	57	3,000,000	1,000	10,000	1,000,000	.....	.....
2-27-'15.....	106	70,000	10,000	1,000	1,000	.....	.....
4-15-'15.....	153	60,000	10,000	1,000	2,000	.....	.....

TABLE V. Cured alfalfa with chloroform.

Date Sampled.	Days.	Bacteriological examination. Quantitative estimation per cc.					
		Plain agar.	Liquefiers in gelatin.	Total acid producers.	Bulgarian group.	Yeasts.	Colon group.
11-13-'14.....	0	10,000,000	.....	1,000,000	.....	100	10,000
11-19-'14.....	6	13,000	1,000	10,000	10	.....	.....
11-25-'14.....	12	10,000	1,000	1,000	20	.....	.....
12- 8-'14.....	25	12,000	1,000	1,000	20	.....	.....
1- 9-'15.....	57	.....	1,000	100	500	.....	.....
2-27-'15.....	106	100,000	1,000	10,000	100	.....	.....
4-15-'15.....	153	10,000	10,000	.....	.....	.....	.....

TABLE VI. Green alfalfa without chloroform.

Date Sampled.	Days.	Bacteriological examination. Quantitative estimation per cc.					
		Plain agar.	Liquefiers in gelatin.	Total acid producers.	Bulgarian group.	Yeasts.	Colon group.
11-12-'14.....	0	20,000,000	10,000	10,000,000	5,000	100	1,000,000
11-19-'14.....	7	1,000,000,000	1,000,000	10,000,000	80,000,000	.....	1,000
11-25-'14.....	13	250,000,000	10,000	100,000,000	80,000,000	.....	.....
12- 8-'14.....	26	87,000,000	100,000	100,000,000	28,000,000	.....	.....
1- 9-'15.....	58	1,000,000	100,000	1,000,000	610,000	.....	.....
2-27-'15.....	107	1,000,000	100,000	1,000,000	2,000,000	.....	.....
4-15-'15.....	154	2,000,000	10,000,000	100,000	20,000,000	.....	.....

Historical Document
Kansas Agricultural Experiment Station

TABLE VII. Green alfalfa with chloroform.

Date Sampled.	Days.	Bacteriological examination. Quantitative estimation per cc.					
		Plain agar.	Liquefiers in gelatin.	Total acid producers.	Bulgarian group.	Yeasts.	Colon group.
11–12–'14.....	0	20,000,000	10,000	10,000,000	5,000	100	1,000,000
11–19–'14.....	7	8,000,000	1,000,000	1,000,000	80	.....	10
11–25–'14.....	13	300,000	100,000	10,000,000	200	100	1,000
12– 8–'14.....	26	.....	1,000,000	1,000,000	.....	.....	.....
1– 9–'15.....	58	3,000,000	100,000	100,000	30	.....	.....
2–27–'15.....	107	10,000,000	1,000,000	1,000,000	100	.....	.....
4–15–'15.....	154	20,000,000	10,000,000	.....	.....	.....	.....

(c) Very good silage was produced from the untreated alfalfa, but the cured alfalfa was probably the best. The silage lacked the sweet aromatic odor common to corn and cane silage. However, the odor was good, but of more acid character.

(d) The treated alfalfa failed to show any characteristic of silage. No silage odor was in evidence, the material showing very little change from the time it was first treated.

(e) The effect of the antiseptic upon fermentation can be noted from the following table showing the acidity of the silage at the conclusion of this experiment.

TABLE VIII.

Material.	Untreated.			Treated.		
	Total acidity.	Nonvolatile as lactic acid.	Volatile as acetic acid.	Total acidity.	Nonvolatile as lactic acid.	Volatile as acetic acid.
Cured alfalfa.....	2.8%	1.57%	1.23%	0.99%	0.78%	0.21%
Green alfalfa.....	2.5%	1.56%	1.24%	0.82%	0.35%	0.47%

Cane silage. The cane used in this experiment was some of the same fodder which was used to fill the small wooden silo previously mentioned. Different percentages of chloroform were used to note the influence of different amounts of chloroform on fermentation. The amounts added were 1, 3, 5, and 10 percents of the total weight of cane.

The results obtained are summarized in tables IX and X.

Historical Document
Kansas Agricultural Experiment Station

TABLE IX.—Cane untreated.

Date Sampled.	Days.	Bacteriological examination.						Chemical examination.			
		Quantitative estimation per cc.						Percent of moisture.	No. cc. N/10NaOH to neutralize acid in 100 gms. silage.		
		Plain agar.	Liquefiers in gelatin.	Total acid producers.	Bulgarian group.	Yeasts.	Colon group.		Total acidity.	Total volatile acids.	Total nonvolatile acids.
2-12-'15	0	30,000,000	10,000,000	10,000,000	100	10,000	1,000,000	66.6	.....	.....	.....
2-13-'15	1	40,000,000	100,000	1,000,000,000	400,000	100,000	1,000,000	65.0	255.75	116.25	139.50
2-15-'15	3	400,000,000	100,000	1,000,000,000	600,000,000	100,000	10,000	65.0	259.90	.....	.....
2-18-'15	6	1,000,000,000	1,000	100,000,000,000	600,000,000	.....	.....	60.0	201.60	.....	.....
2-20-'15	8	3,000,000,000	10,000	1,000,000,000	100,000,000	.....	.....	65.0	203.40	.....	.....
2-23-'15	11	2,000,000,000	100,000	1,000,000,000	500,000,000	.....	.....	67.0	315.22	152.18	163.04
3- 4-'15	20	110,000,000	1,000	10,000,000	40,000,000	.....	.....	70.0	353.40	153.90	199.50
3-20-'15	36	200,000	1,000	1,000,000	2,000	.....	.....	60.0	324.80	134.40	190.40
4-12-'15	59	10,000	1,000	100,000	200,000	.....	.....	85.0	317.55	153.30	164.25
5- 5-'15 z	83	400,000	10,000	100,000	10,000	70,000	.....	85.0	358.05	173.60	184.45

Historical Document
Kansas Agricultural Experiment Station

TABLE X. *Cane + 1 percent chloroform.*

Date Sampled.	Days.	Bacteriological examination. Quantitative estimation per cc. Plain agar.	Liquefiers in gelatin.	Total acid producers.	Bulgarian group.	Yeasts.	Colon group.	Chemical examination. Percent of moisture.	No. cc. N/10 NaOH to neutralize acid in 100 gms. silage. Total acidity.
3- 6-'15....	22	40,000	100,000	.....	.....	.....	.....	60.0	74.2
3-20-'15....	36	.....	1,000	100,000	.....	.....	.....	45.0	62.7
4-12-'15....	59	15,000	100,000	100,000	.....	.....	.....	72.7	53.6
5- 5-'15....	83	3,000	1,000	10,000	.....	.....	.....	70.0	64.90
Cane + 3 percent chloroform.									
3- 6-'15....	22	10,000	1,000	1,000	.....	.....	.....	70.0	64.0
3-20-'15....	36	.....	10,000	10,000	.....	.....	.....	54.0	52.70
4-12-'15....	59	13,000	1,000	10,000	.....	.....	.....	66.5	74.37
5- 5-'15....	83	3,000	1,000	100,000	.....	.....	.....	75.0	65.23
Cane + 5 percent chloroform.									
3- 6-'15....	22	20,000	1,000	1,000	.....	.....	.....	75.0	64.5
3-20-'15....	36	.....	100,000	1,000	.....	.....	.....	55.0	52.75
4-12-'15....	59	3,000	1,000	10,000	.....	.....	.....	67.7	64.26
5- 5-'15....	83	50,000	1,000	1,000,000	.....	.....	.....	80.0	75.60
Cane + 10 percent chloroform.									
3- 6-'15....	22	10,000	1,000	1,000	.....	.....	.....	77.7	64.6
3-20-'15....	36	10,000	1,000	1,000	.....	.....	.....	40.0	52.0
4-12-'15....	59	100,000	1,000	10,000	.....	.....	.....	75.0	53.75
5- 5-'15....	83	50,000	1,000	100,000	.....	.....	.....	65.0	63.90

It does not appear that any additional remarks on tables IX and X are necessary, as the summary is very similar to the preceding data mentioned. The untreated cane fermented into a very good grade of silage, while all of the treated samples showed.no trace of silage fermentation. It appears from these results that an addition of one percent chloroform is as efficient as a greater amount in checking microbial growth.

Historical Document
Kansas Agricultural Experiment Station

THE PREDOMINATING TYPES OF MICROÖGANISMS FOUND.

From the morphological, cultural and biochemical study of the numerous cultures isolated from different silages at different stages of fermentation, the following groups were differentiated:

1. Miscellaneous type.
2. Yeasts.
3. Colon group.
4. Acid group.

The miscellaneous type includes those microörganisms which apparently had no influence in the normal ripening process, in that there was no definite predominating type common to this group. They were always present on the forage plant as it went into the silo, and at this time only did they show any importance as to numbers. However, this includes several important species that would produce harmful fermentations if the proper conditions were present. Probably the two most important classes of this group would include the molds and the spore-producing, rod-shaped, gelatin-digesting bacteria.

Yeasts are a group of microörganisms found in silage, and, no doubt, are influential in its ripening. They are, in most cases, present in large numbers on the forage plant as it goes into the silo. Usually the maximum number is reached within the first five days. Examinations of some silages showed no increase in their numbers from the start; however, in most cases a small to large increase was usually noted. A gradual decrease in these numbers is observed soon after they reach their maximum number until they finally disappear. Numerous yeast cultures have been isolated throughout this work, but very little time has been spent in identifying and differentiating them. Most of the efforts have been devoted to determining the relative number of organisms of the different groups present at the various stages of fermentation, rather than to a detailed study of the individual species present. It has been observed, however, that the same types of yeast have always been present in all stages examined and likewise the same group predominated throughout the fermentation. Whether this is due to the medium employed, which favors only these types, must be left for future study.

The colon group is another class of important organisms which has always been found in all kinds of silage studied. The results seem to indicate that the yeast and colon fermentation follow each other closely throughout the process. Only

Historical Document
Kansas Agricultural Experiment Station

the total number of colon organisms as a group has thus far been determined. The differentiation of this group into species would not be of much importance from a practical standpoint. However, it would, no doubt, be of scientific interest in so far as the fermentation is concerned.

As stated before, the acid group, common to all classes of silage, is, from the standpoint of desirability, the most important group of microörganisms found in silage. The group can be divided into two types—the low acid-producing bacteria, similar to the common *Bacterium lactis acidi,* and the high acid-producers, which evidently would be classified with the Bulgarian group. The low acid-producers are usually found in the very early stages of fermentation and in fewer numbers than the high acid-producing type.

The Bulgarian type is the predominating organism found in the fermentation of normal silage. Its numbers are low on the forage plant as it goes into the silo; in most cases lower than any other group. It reaches its maximum numbers within the first few days after filling the silo, but a gradual decrease is eventually observed, although not until long after the material could be called silage.

A more detailed study of this group of bacteria was made, and several hundred 'cultures have been isolated and studied. Some of their principal characteristics follow, but a more complete study of this group will be published in the future.

MORPHOLOGY OF THE BULGARIAN GROUP.

Morphologically, much difference was noted between the various cultures isolated, while marked deviations occurred in the same culture. In size, the organisms vary from small oval rods to much larger, more defined rods. The majority of all cultures studied showed the characteristic filaments and granular staining, the filaments of different cultures varying in length and in diameter. It appeared characteristic for some cultures to have long filaments.

Stained preparations from young cultures almost invariably showed the small oval rods, while preparations from the same cultures a few days later showed the filaments to be present in large numbers, and the granular staining was more pronounced. Bifid types were never observed, although the curved and spiral-like filaments were numerous. Stained preparations from agar slant cultures usually possessed these small rods and often showed the short filaments arranged in a parallel manner. Organisms with a single curve were ob-

Historical Document
Kansas Agricultural Experiment Station

served from the same culture medium. No motile forms were recorded.

CULTURAL AND BIOCHEMICAL FEATURES OF THE BULGARIAN GROUP.

The colonies of this Bulgarian group vary greatly in size and form of growth. In size, the colonies vary from very minute forms, scarcely visible to the naked eye, to colonies as large as the common lactic acid colony or a little larger.

The form of the colonies seems to vary greatly. The characteristic ‘‘woolly edge” colony was frequent; however, the predominating colonies were very similar to the common lactic acid types, either lance shaped or small and dense with a uniform edge. A zone of cloudiness or haziness, which included the colony, was characteristic of this type. The colonies appeared at both the surface and in the deep agar. The fact that the colonies from this group of organisms are frequently very similar to the common *Bacterium lactis acidi* colony is, no doubt, the reason why this group has been overlooked by previous workers. The growth on plain agar slants is very slight, but usually present; however, on glucose agar slants the growth is very good, the characteristic growth being beaded to effuse. The same growth tendency is noted in nutrient broth (a sugar hastens growth). From observations of the three sugars, dextrose, lactose and saccharose, it seems that the former is preferred. In glucose broth the organisms produce a moderate to heavy clouding, together with a viscid or flocculent sediment.

Litmus milk is coagulated and litmus reduced. At first the time of coagulation varies from two to fourteen days when incubated at 35° C. After repeated transfers, however, coagulation is more prompt. With the addition of peptone in the milk, coagulation is hastened, taking place within two to four days, usually, at incubator temperature.

The organisms isolated directly from colonies on plate cultures to glucose litmus broth and litmus milk, respectively, show a difference in regard to their rate of development. A vigorous growth with acid reaction is observed in the broth culture much before the acid reaction and coagulation is apparent in the milk culture. In the broth culture a good growth is observed within one to four days at 35° C., while in litmus milk the growth is much slower.

Historical Document
Kansas Agricultural Experiment Station

The total acid producers recorded from the glucose litmus broth will, in the majority of cases, give a truer estimate of the Bulgarian group present in the silage than those of the acid agar media. The numerous microscopic examinations made directly from the litmus broth tubes showing acid reaction in the highest dilutions, always exhibited these organisms in large numbers and in practically pure culture. Pure cultures were always isolated from these tubes when desired.

The amount of acidity produced in milk by the different cultures varies from about 0.9 to 2.5 percent calculated as lactic acid. The rate of acid production differs in plain and peptone milk. The final percent of acidity is practically the same, but the maximum acidity is attained more rapidly in peptone milk than in the plain milk cultures.

Table XI shows the degree and rate of acid development when growing in plain and peptone milk at a temperature, of 35° C.

TABLE XI. Action of Bulgarian cultures in plain and peptone milk. (Figures give number cc. of N/20 NaOH required to neutralize the acid in 5 cc. of milk, after the acidity of the check has been deducted from each.)

Cultures.	5 days.		10 days.		15 days.		20 days.		25 days.		30 days.		35 days.	
	Plain milk.	Peptone milk.	Plain milk.	Peptone milk.	Plain milk.	Peptone milk.	Plain milk.	Peptone milk.	Plain milk.	Peptone milk.	Plain milk.	Peptone milk.	Plain milk.	Peptone milk.
65S....	1.6	7.3	2.0	8.6	4.5	12.9	4.7	13.1	5.5	16.0	7.9	16.0	9.9	18.4
2X...	1.3	7.3	1.6	8.8	2.5	9.0	1.5	8.4	2.2	9.2	8.1	11.4	7.7	13.3
44S....	2.0	7.0	1.1	8.6	5.2	10.4	6.3	11.7	9.2	12.1	8.5	13.7	8.5	18.5
8S....	3.1	1.9	1.5	0.7	1.8	1.7	0.2	1.6	0.2	2.9	0.5	3.6	1.5	.....
9S....	4.1	1.9	1.5	2.0	3.4	4.1	2.5	5.0	4.0	5.1	6.1	5.4	8.2	8.7
29S....	2.9	2.6	0.1	0.7	2.9	1.1	0.7	1.5	3.1	2.9	4.7	3.2	9.3	6.2
66S....	5.7	9.1	5.7	10.6	7.3	15.2	8.1	15.4	11.8	15.1	11.8	20.3	16.5	25.9
70.....	2.3	7.3	1.5	8.7	4.6	10.4	4.8	12.1	7.8	13.5	9.5	14.9	11.4	16.7
B14...	2.9	5.7	2.0	8.2	4.8	12.1	5.7	12 5	8.0	12.4	8.3	16.3	10.6	20.4
90S....	2.8	9.3	3.8	11.0	5.7	12.2	6.2	11.9	8.2	13.2	9.2	14.9	11.9	15.7
96.....	3.7	10.2	4.8	9.4	7.3	10.3	7.3	9.8	9.4	12.8	12.0	13.9	14.0	17.6
90.....	3.4	10.0	3.3	10.9	6.4	12.5	5.6	13.2	6.6	13.9	7.9	13.7	11.3	17.6
28S....	4.9	8.3	3.6	7.9	4.4	7.1	3.7	6.7	5.9	8.8	5.3	11.4	9.5	14.0
61S....	1.8	4.9	1.2	7.6	3.8	9.2	3.4	11.9	3.9	15.3	4.6	15.0	7.3	15.7
2A...	2.8	2.8	0.1	0.5	1.5	0.7	1.3	4.0	2.5	4.4	3.7	4.2	8.5	8.8
B12...	2.3	10.4	5.2	11.4	9.0	11.9	10.3	12.6	12.5	15.3	12.5	11.6	15.5	17.8
27S....	2.2	1.9	0.5	0.4	2.2	0.9	0.7	2.4	1.5	1.2	2.2	2.7	6.5	4.9
4A....	1.7	1.7	0.1	1.4	0.7	5.1	0.6	7.4	2.0	8.1	3.1	9.5	6.5	11.0

Historical Document
Kansas Agricultural Experiment Station

CHIEF FERMENTATIONS IN SILAGE.

Three important fermentation changes thus far observed in the ripening of the ensilage are:

1. The fermentation by yeasts.
2. The fermentation by the colon group.
3. The fermentation by the acid-producing group.

As mentioned previously, the yeast and colon fermentations are the first fermentations noted and appear to develop and disappear almost simultaneously. Their exact importance can not be stated at this time. However, much of the sweet aroma common to good silage is the result of yeast fermentation. While the effect of colon fermentation in silage production is undecided, it is evident that it should be considered. The presence of large numbers of this group at a very important stage of ripening is sufficient to place it under observation. Although the data obtained are not sufficient to warrant a positive statement, it seems that a portion of the volatile acids found in silage results from this colon fermentation.

The products which result from the fermentation of different silages may differ widely on account of the different kinds of forage used and the varying conditions under which they are siloed. Although all silage may be eaten readily by stock and be nutritious, some will, no doubt, exhibit characteristics which must make them of better grade than others. What these desired characteristics are, and how obtained, can only be determined by an exhaustive study of the fermentation changes. No doubt the important factors which will best determine a good silage are: (1) the acid content; (2) the odor; (3) the feeding value.

Acid production in silage is a most desirable and essential factor. Its importance can be attributed to the preservative effect which it offers to the silage as well as its probable ability of increasing the palatability of the feed. The present investigation warrants the statement that acid production, common to all normal silage, is largely the result of fermentation by the Bulgarian group of bacteria.

The acids of silage can be divided into volatile and nonvolatile groups. The latter acids are represented chiefly by lactic acid, while the larger part of the volatile acids is normally acetic acid. As previously mentioned, the nonvolatile acid usually predominates. The varying conditions in the methods and the different materials used for silage make it unsafe definitely to state the exact ratio between the volatile

Historical Document
Kansas Agricultural Experiment Station

and nonvolatile acids of silage. However, the ratio between the two is close to that already mentioned (1.0 to 0.75 in favor of the nonvolatile acids) for all normal and good silages. A marked increase or decrease in this ratio is generally followed by a decrease in the desirable quality of the aroma and probably in the palatability of the silage.

The source of the acetic acid in silage is still an unsettled question. It seems evident that by far the greater part of the acid production in silage is dependent upon the fermentation of carbohydrates. Previous investigators have mentioned the oxidation of alcohol as a probable source of acetic acid, the alcohol being a result of yeast fermentation. The insufficient amounts of oxygen and alcohol normally present in the fermenting material, fail to account for such large amounts of acetic acid as are found in most silage and make one doubt this theory.

A probable source of the acetic acid in silage and the factors concerned in its production are suggested by the results of a few preliminary experiments. It should be understood, however that the comparatively small number of examinations make it impossible to draw definite conclusions. Several organisms may be influential in acetic acid production, but the Bulgarian bacilli seem the leading group to which this characteristic can be attributed. Hart, Hastings, *et al.* (8) in the analysis of the products formed in milk cultures of *Bacterium casei,* found acetic acid to be present in large amounts as the principal volatile acid. The average ratio of the nonvolatile to the volatile acids formed was 1.0 to 0.46 for the two cultures analyzed. *Bacterium casei* and *Bacterium bulgaricus* are synonyms for the same group of organisms, according to these authors.

With this fact in mind, sterile milk containing one percent peptone was inoculated with different cultures of the Bulgarian group isolated from silage. The flasks of milk were sealed with paraffin to prevent evaporation and incubated at 35° C. for forty-two days. The total acidity and volatile acids were determined from each culture flash. The results of analysis are given in table XII.

Alfalfa extract, obtained from the juice of the freshly cut plant by pressure was sterilized and inoculated with pure cultures of the *Bulgarian, colon* and *yeast* organisms. One percent of glucose was added to the extract to furnish additional

Historical Document
Kansas Agricultural Experiment Station

TABLE XII. Acidity produced by the Bulgarian group. (Computed as number of cc. of N/10 alkali required to neutralize the acidity in 100 cc. of milk.)

Culture.	Total acidity.	Total nonvolatile acidity.	Total volatile acidity.	Ratio of nonvolatile to volatile acids.
61S	206.0	156.0	50.0	1.0—0.32
124	192.0	132.0	60.0	1.0—0.46
B14	250.0	193.0	57.0	1.0—0.29
125	194.0	164.0	30.0	1.0—0.18
96	190.0	133.0	57.0	1.0—0.43
144	214.0	169.0	45.0	1.0—0.26
Average	207.66	157.83	49.83	1.0—0.31

Note.—This ratio would be a trifle less if it had been determined on the basis of the percentage of lactic acid to the total percentage of volatile acids present, as was done by Dox and Neidig (6-7). Instead, the ratio was determined by using the number of cc. of N/10 alkali to neutralize the volatile and nonvolatile acids.

carbohydrates. Cultures of the above organisms were used in combination to inoculate the flasks. The cultures were sealed to prevent evaporation and allowed to incubate three months at 35° C. The results of the analysis are found in table XIII.

TABLE XIII. Acidity in alfalfa extract. (Computed as number of cc. of N/10 alkali required to neutralize 100 cc. of extract.)

Cultures.	Total acidity.	Total nonvolatile acidity.	Total volatile acidity.	Ratio of nonvolatile to volatile acids.
Bulgarian flask (1)	370.0	213.0	157.0	1.0—0.73
Bulgarian flask (2)	290.0	188.0	102.0	1.0—0.54
Colon	120.0	63.0	57.0	1.0—0.90
Bulgarian + yeast (1)	164.0	104.0	60.0	1.0—0.57
Bulgarian + yeast (2)	178.0	98.0	80.0	1.0—0.81
Bulgarian + yeast + colon (1)	275.0	171.5	103.5	1.0—0.60
Bulgarian + yeast + colon (2)	225.0	120.0	105.0	1.0—0.87

The carbon dioxide, which was evidently present in several of the cultures, as a result of fermentation, would influence the determination of the volatile acids. It was removed by passing carbon-dioxide-free air through each culture flask for two hours at a temperature of 25° C.

The summary of this small number of analyses indicates the ability of the Bulgarian group to produce volatile acids. The larger percent of the volatile acids produced, is acetic acid according to the analyses of a few cultures by Hart, Hastings.

Historical Document
Kansas Agricultural Experiment Station

et al. (8). It therefore appears perfectly logical, from these results, to assume that this group would produce acetic acid while growing in silage. Although these organisms evidently do not produce all of the acetic acid found in normal silage, they must be responsible for a large percent of it. The balance of the volatile acids, almost always present, could easily be accounted for as due to the result of other fermentations. The colon organisms are no doubt instrumental in the production of a considerable amount of these volatile products. Likewise, a small amount of volatile acids may be produced through proteolysis by miscellaneous organisms, but very small amounts of the volatile acids are derived from this source in normal and good silage. The direct decomposition of carbohydrate material by single groups of organisms seems to be a more plausible source of such acids.

BIBLIOGRAPHY.

1. BABCOCK, S. M., and RUSSELL, H. L. 1903. "Causes Operative in the Formation of Silage." Twentieth Annual Report, Wisconsin Experiment Station, p. 243.

2. RUSSELL, E. J. 1908. "Chemical Changes taking Place During the Ensilage of Maize." Journal of Agricultural Science, vol. 2, No. 4, p. 392.

3. SAMARANI, FRANCO. 1913. "The Preparation of Ensilage." Bollettino del Ministero di Agricultural Industria e Commercio, year 13, Nos. 8-12, pp. 87-103. (Abstract from International Institute of Agriculture.) Bureau of Agricultural Intelligence and Plant Diseases, Year 5, No. 12, p. 1625.

4. GORINI, C. 1911. "Bacteriological Research on Ensilage Forage." Annuaraio delta Instituzione Agraria Dott. Andrea Ponti, vol. II, pp. 165-179. (Abstract from International Institute of Agriculture.) Bureau of Agricultural Intelligence and Plant Diseases, Year 5, No. 6 p. 785.

5. ESTEN, W. M., and MASON, CHRISTIE J. 1912. "Silage Fermentation." Storrs Agricultural Experiment Station Bulletin No. 70, p. 40.

6. DOX, ARTHUR W., and NEIDIG, RAY E. 1913. "The Volatile Aliphatic Acids of Corn Silage." Iowa Agricultural Experiment Station. Research Bulletin No. 7, pp. 4-32.

7. DOX, ARTHUR W. and NEIDIG, RAY E. 1913. "Lactic Acid in Corn Silage." Iowa Agricultural Experiment Station. Research Bulletin No. 10, pp. 365-378.

8. HART, E. B., HASTINGS, E. G., FLINT, E. M., and EVANS, ALICE C. 1914. "Relation of the Action of Certain Bacteria to the Ripening of Cheese of the Cheddar Type." Journal of Agricultural Research, vol. 2, No. 3, pp. 193-203.