The quality of hay-crop silage has often been related to the chemical composition of the finished product, but detailed investigations on the step-wise chemical changes occurring during the fermentation have seldom been reported. Conversely, the relationship between silage quality and the changing bacterial spectrum is not clearly understood. Although the bacterial changes during the storage period have been plotted by several workers, the significance of their results has been difficult to determine because simultaneous chemical studies were not included. Furthermore, most experiments have been conducted on miniature or experimental sized silos, and it has never been proved that these results are applicable to the fermentations occurring in farm sized silos.

Consequently, the main object of the present investigation was to interrelate silage quality with both the chemical and bacterial changes which occur when grasses and legumes are ensiled in full-sized silos on typical farms. Since this study was completed, Langston et al. (1958) have published the results of similar investigations with experimental sized silos.

The chemistry of forage-crop silage has been comprehensively reviewed by Barnett (1954). It has been repeatedly confirmed that when well preserved silages are compared to spoiled silages the former have low pH values, large amounts of lactic acid, and small amounts of butyric acid and volatile base.

The lactic acid bacteria are the most important agents of silage preservation, although they are not the only bacteria present in normal fermentations (Burkey et al., 1953). Kroulik et al. (1955a, b) found very few lactic acid bacteria on fresh crops, but normally there were as many as $10^4$ to $10^6$ per g within a day or two after ensiling. Although homofermentative rods carried out the main lactic fermentation, pediococci and streptococci were observed in the early stages of the fermentation, and heterofermentative rods predominated later in the storage period. The population of lactic acid bacteria can be modified by wilting (Kroulik et al., 1955b), mincing (Stirling, 1951), and other factors (Barnett, 1954).

Beynon and Pette (1936) were the first to attribute the butyric acid associated with spoiled silage to the lactate-fermenting activity of Clostridium tyrobutyricum. The characteristics of this organism have been detailed recently by Bryant et al. (1952). Unfortunately, all reports of clostridia have been based on spore counts. Rosenberger (1951) cautioned against the use of spore counts to measure lactate-fermenting activity, since these organisms do not form spores during the stage of their most rapid development, but only after active growth ceases. In our study, lactate-fermenting anaerobes were detected in the vegetative state.

The course of the fermentation is undoubtedly influenced by factors such as the plant species, the moisture content, and the maturity of the crop; but the relative importance of each factor has not been clearly defined. The silages studied for this report were prepared under a wide range of field conditions. Weather data were collected and details of the harvesting procedure were recorded in an attempt to relate silage quality to environmental conditions at the time of ensiling.

**Experimental Methods**

The silages were prepared by individual farmers on randomly selected farms in Michigan’s Eaton and Ing-
ham counties. Before harvesting, the amount of each plant species in the crop mixture and the quality of the stand were estimated. At the time of harvesting, the date, time of day, temperature, relative humidity, and the interval since the last rainfall were recorded. The amount of cloud cover was estimated. Details of the harvesting procedure were noted, such as whether the crop was swathed and whether wilting had taken place. The size and type of silo were also recorded.

Temperature profiles of the top 6 to 7 ft of silage were obtained by inserting thermocouples at intervals of 1 ft. Profiles were obtained only for representative silages, and only twice during the storage period, but the temperature of each sample was recorded with a thermometer immediately before sampling.

**Sampling methods.** Samples of the fresh plant material were taken at the instant the forage was being fed into the silo, at a time when the silo was about 3 ft from being full. In upright silos, subsequent samples of the fermenting silage were taken at a mean depth of 3 ft so that the silage samples would correspond to the samples of fresh forage. In the more tightly packed bunker silos, samples were taken at a depth of 1 ft. Silage samples were taken at least twice in the first 10 days after ensiling and at approximately 2-week intervals thereafter during the storage period.

Silage quality was estimated at the time of sampling on the basis of general appearance and odor. It was found that most silages could be classified either as well preserved or obviously spoiled. Where insufficient care was taken in packing, the silage became grossly overheated to a depth of as much as 12 ft, as evidenced by temperatures in excess of 125 F. Since this occurred all too frequently, a study of the changes occurring in this overheated layer was included. The results of subsequent chemical and bacterial analyses showed that the silages within the well preserved, spoiled, and overheated groups were reasonably similar, but that the groups were remarkably different. Of 27 silages observed, only 1 had to be classified as "intermediate" in quality.

In the upright silos it is recognized that a sample from a depth of 3 ft cannot be considered as representing the entire silage. However, when the silage was adequately packed, temperature readings indicated that the silage at this depth was not subject to the rise in temperature which was characteristic of the surface layers. It was possible to obtain samples from varying depths from the relatively shallow bunker silos, but analyses of several samples taken from other depths indicated that samples from a depth of 1 ft were representative of the silo.

All samples were packed in 1 qt polyethylene freezer bags, sealed, and transported to the laboratory in a portable chest containing Dry Ice.

**Bacteriological analyses.** A finely cut, 10-g portion of each sample was placed in a Waring Blender with 90 ml of isotonic saline, and agitated for 10 min at a slow speed. Suitable dilutions were made for the preparation of culture plates. The remainder of the sample was resealed, quick frozen and stored at -4 F for future chemical determinations. This procedure is analogous with that developed by Kroulik et al. (1955a).

The lactic acid bacteria were enumerated on LBS medium (BBL) and counts of the "total" number of organisms capable of growing anaerobically were obtained by the use of Brewer plates and anaerobic agar containing glucose and Eh indicator (BBL). The interval between sampling and the completion of plating rarely exceeded 6 hr.

Representative isolates from the anaerobic agar were purified by further passage through tubes of the same medium. Those isolates which proved to be obligate anaerobes by their zone of growth in stab cultures were tested for proteolytic and lactate-fermenting ability in the media designed by Rosenberger (1951).

The morphological and fermentative characteristics of 542 isolates from LBS medium were also recorded.

**Chemical analyses:** The samples were cut into 1/4-in. lengths with hand scissors while in a semifrozen state and immediately refrozen. The extractions for organic acids and volatile bases were begun while the samples were still frozen.

The \( \mu \) moles of butyric, propionic, acetic, formic, lactic, and succinic acids per g of fresh matter were determined chromatographically by the method of Wiseman and Irwin (1957).

Volatile bases and amino acids were measured by the Woodman (1925) modification of the Foreman method. The experimental details of this procedure have been outlined by Barnett (1954). Extracts were made in 2-L screw-cap bottles on a shaker which reciprocated through 5 in., 75 times per min. Results were expressed as \( \mu \) moles of acid or base per 100 g of fresh matter by an adaptation of the formula given by Watson and Ferguson (1937).

To determine the pH, 3 to 5 g of material were added to 20 ml of distilled water in a 50-ml beaker. The mixture was stirred and the pH was measured with a Beckman\(^*\) model G pH meter.

The moisture content was calculated from the weight loss of 5-g samples which had been heated in a dry oven at 221 F for 24 hr. In addition, the amount of carbohydrate was determined by a modification of the phenol-sulfuric acid method developed by Koch et al. (1951), and protein was calculated from values obtained by the Kjeldahl nitrogen technique.

**Results**

Complete analytical data for individual silages have been given elsewhere (Kempton, 1958). Originally, observations were made on 27 different silage batches.

---

\* Baltimore Biological Laboratory, Inc., Baltimore, Maryland.

\* Beckman Instruments, Inc., Fullerton, California.
The results presented here have been drawn from 13 silages which did not receive any pretreatment or preservatives and on which the most complete observations were made. Of the 13 silage batches, six were in the well preserved class, five were spoiled, and two had been excessively overheated. A general description of these silages is given in table 1. Each graph has been drawn from the data of only one silage, but similar results were obtained from every other silage in the respective quality group, except where specifically noted.

After 3 weeks in the silo, the temperature in an overheated silage was still 138 °F at sampling depth, as shown in table 2. This entire batch was overheated since the silage was only 7 ft deep. Temperature in the upper layers of two upright silos shows the effect of different packing procedures, but both of these were “well preserved” at sampling depth. Covering the silage was more effective than trampling in preventing overheating. There was less fluctuation in the more tightly packed bunker silos but, after being ensiled 3 weeks, there was no appreciable temperature difference between spoiled and well preserved silages.

The accumulation of organic acids in a typical, well preserved silage is given in figure 1a. All other well preserved silages showed comparable, steady increases in lactic acid and a small constant level of succinic acid. Formic acid was present in trace amounts in all samples but butyric and propionic acids were never found. In all the spoiled silages (figure 1b) there was an initial lactic fermentation, but the lactic acid was later replaced by butyric and propionic acids in an approximate ratio of 2:1. Figure 1c indicates that the overheated silages underwent an acetic fermentation for the most part, although a small amount of lactic acid did accumulate.

The amount of acetic acid was not related to quality. All silages studied, regardless of quality, contained maximum acetic acid concentrations in the relatively narrow range of 88 to 213 μmoles per g. Peak acetic acid concentration was reached earlier in some silages than in others, but this bore no relationship to quality.

The effect of acid production on the pH changes in well preserved and spoiled silage is given in figure 2.

The accumulation of amino acid and volatile base in a well preserved silage and a spoiled silage is compared in figure 3. A substantial accumulation of amino acid accompanied by a small amount of volatile base was observed in all well preserved silages and was also found in both overheated silages. Compared to well preserved silages, all spoiled silages exhibited a considerably greater production of amino acid soon after ensiling. In all but one of the spoiled silages, a large amount of volatile base was produced at the expense of amino acid in the later periods of storage. Silage 10 was classified as “spoiled,” but excessive volatile base was not produced although all the lactic acid had been replaced by butyric and propionic acid.

Examples of the number of silage bacteria capable of growing on LBS medium at different periods of storage are shown in figure 4. In the first few days after ensiling, a rapid multiplication of lactic acid bacteria occurred in both well preserved and spoiled silages. After a gradual decline in numbers over the next 3 to 4 weeks, 75 per cent of the well preserved and spoiled silages showed a secondary increase in lactic acid bacteria such as is pictured in figure 4. The overheated silages were characterized by the failure of the lactic acid bacteria to increase over the number present on the fresh crop. Isolates from LBS medium included pediococci, diplo- rods, and long rods with a wide range of fermentative

### TABLE 1

**General description of crops and silages**

<table>
<thead>
<tr>
<th>Quality Group</th>
<th>Silo No.</th>
<th>Silo Type</th>
<th>Date Filled</th>
<th>Crop</th>
<th>Quality of Crop</th>
<th>Final pH</th>
<th>Composition at End of Storage Period: Fresh Matter (μmoles/g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lactic acid</td>
</tr>
<tr>
<td>Well preserved</td>
<td>1</td>
<td>Upright</td>
<td>June 26</td>
<td>June clover</td>
<td>Excellent</td>
<td>4.4</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Upright</td>
<td>June 21</td>
<td>Alfalfa, brome</td>
<td>Excellent</td>
<td>4.1</td>
<td>245</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Upright</td>
<td>July 16</td>
<td>Oats</td>
<td>Excellent</td>
<td>4.4</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Upright</td>
<td>July 3</td>
<td>Alfalfa, alsike</td>
<td>Excellent</td>
<td>5.2</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Upright</td>
<td>June 29</td>
<td>Alfalfa</td>
<td>Good</td>
<td>4.4</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Upright</td>
<td>July 3</td>
<td>Mammoth clover</td>
<td>Excellent</td>
<td>4.3</td>
<td>156</td>
</tr>
<tr>
<td>Spoiled</td>
<td>7</td>
<td>Bunker</td>
<td>June 8</td>
<td>Alfalfa</td>
<td>Excellent</td>
<td>5.9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Bunker</td>
<td>June 9</td>
<td>Alfalfa</td>
<td>Excellent</td>
<td>5.8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Bunker</td>
<td>June 14</td>
<td>Alfalfa, brome</td>
<td>Excellent</td>
<td>5.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Bunker</td>
<td>July 2</td>
<td>Alfalfa</td>
<td>Excellent</td>
<td>5.1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Bunker</td>
<td>June 20</td>
<td>Alfalfa, alsike</td>
<td>Good</td>
<td>5.8</td>
<td>1</td>
</tr>
<tr>
<td>Overheated</td>
<td>12</td>
<td>Upright</td>
<td>June 21</td>
<td>Alfalfa</td>
<td>Poor, weedy</td>
<td>4.4</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Upright</td>
<td>June 18</td>
<td>Alfalfa</td>
<td>Fair</td>
<td>5.0</td>
<td>29</td>
</tr>
</tbody>
</table>
abilities. However, there was no relationship between the type of lactic acid bacteria and silage quality.

Similarly, there was no consistent difference in the count of “total anaerobes” between spoiled and well preserved silages (figure 5).

However, when colonies picked from the BBL anaerobic medium were tested in Rosenberger’s (1951) media, essentially all the isolates from spoiled silages resembled C. tyrobutyricum insofar as they produced butyric acid from lactate but did not liquefy gelatin. Conversely, the bulk of the “total anaerobes” in well

### TABLE 2

Silage temperatures at different depths 3 weeks after ensiling

<table>
<thead>
<tr>
<th>Depth</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spoiled (silo 7)</td>
</tr>
<tr>
<td>ft</td>
<td>F</td>
</tr>
<tr>
<td>1/2</td>
<td>104</td>
</tr>
<tr>
<td>1</td>
<td>104</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>114</td>
</tr>
<tr>
<td>4</td>
<td>112</td>
</tr>
<tr>
<td>5</td>
<td>112</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
</tr>
</tbody>
</table>

![Figure 1](http://aem.asm.org/)  
**Figure 1.** The production of organic acids in typical silages of different quality.

![Figure 2](http://aem.asm.org/)  
**Figure 2.** Comparison of pH changes in a well preserved silage and a spoiled silage.

![Figure 3](http://aem.asm.org/)  
**Figure 3.** Comparison of the production of amino acid and volatile base in a typical well preserved silage and a spoiled silage.
preserved silages proved to be lactic acid bacteria. With fresh forages, the high counts on anaerobic agar compared to LBS medium were due to the facultative growth of the chromogenic bacteria which comprise the predominant flora of the fresh plant.

If a silage was going to spoil, vegetative cells of lactate-fermenting clostridia were detected by this procedure within a day after ensiling, even before butyric acid had appeared.

Unfortunately, it could not be determined why lactate-fermenting anaerobes proliferated in some silage and not in others. Although all the spoiled samples came from bunker silos, there was no evidence to suggest an inherent fault in this type of silo. Silage quality could not be related to any environmental factor at the time of ensiling, such as weather conditions, crop mixtures, or the amount of moisture, protein and fermentable carbohydrate in the fresh plants.

Discussion

The silages were not sampled at identical intervals during the storage period because of the physical limitations to sampling a large number of silos located as much as 30 miles from the laboratory. It was, therefore, impossible to plot average values, but it was found that the general trends in the silage fermentation could be satisfactorily illustrated by the data of only one silage from each quality group.

For both well preserved and spoiled silages, the pattern of organic acid production in full-sized silos proved to resemble closely the pattern obtained by Langston et al. (1958) from their study of experimental sized silos. In the first few days after ensiling, lactic acid was formed in all silages which had not been excessively over-heated. Spoilage occurred when the lactic acid was subsequently replaced by butyric and propionic acids.

From figure 1b it is evident that the large amount of butyric and propionic acid present at the end of the storage period could not have been derived solely from the lactic and succinic acid produced in the initial stages. Either lactic acid was being formed throughout the storage period and was immediately converted to butyric and propionic acids, or some of the spoilage acids were derived directly from plant carbohydrate.

An appreciable amount of acetic acid was found in all silages, including those which were overheated, but neither the final amount of acetic acid nor its pattern of accumulation was related to silage quality. The production of acetic acid could not be directly linked to the population of any of the bacterial groups studied.

Contrary to what was expected, in one spoiled silage the butyric fermentation was not accompanied by the production of large amounts of volatile base. Langston et al. (1958) used similar discrepancies as the basis for classifying a group of silages as “intermediate” in quality. Evidently the conditions which cause these two different aspects of silage are not directly linked. Bender et al. (1941) suggested that the production of volatile base takes place when the supply of fermentable carbohydrate has been exhausted and amino acid is utilized as a source of carbon. In this experiment, the results of carbohydrate analyses were inconclusive.

The number of lactic acid bacteria was approximately the same in both well preserved and spoiled silages at every stage of the fermentation, which is again in agreement with the work of Langston. The population of lactic acid bacteria reached a peak a few days after ensiling and subsequently declined. Later in the storage period, the number of lactic acid bacteria usually underwent a further slight increase such as Kroulik et al. (1955b) had observed. The similarity between the lactic-acid bacteria in well preserved and spoiled silages is further evidence that inoculation with these bacteria would be useless.

Langston et al. (1958) were able to demonstrate a sequence of morphologically and physiologically differ-
ent types of lactic acid bacteria during the fermentation, and show some relationship between silage quality and the relative number of each type. These results were not verified, perhaps due to the wider variation in the kind of silage or the relatively fewer samplings.

An attempt was made to demonstrate the presence of lactate-fermenting anaerobes in the vegetative state. Since the medium used was not selective, the lactate-utilizing bacteria could not be detected unless they were present in predominant numbers. However, since almost all the bacteria isolated from spoiled silages with this medium were found to ferment lactate, the "total" anaerobic count in spoiled silages becomes essentially a count of lactate-fermenting anaerobes. Although the total count was not significantly different in well preserved silages, only the occasional lactate-fermenting organism was found. The anaerobic population of good silages was composed almost exclusively of lactic acid bacteria. Similarly, the anaerobe count on fresh forages proved to be a count of the typical chromogenic bacteria of fresh plant material which were able to grow facultatively.

Without a selective medium it is impossible to state whether silages which spoiled contained increased numbers of lactate-fermenting organisms at the time of ensiling. It was found that whenever spoilage occurred these organisms multiplied rapidly soon after ensiling and reached a peak within a few days. Conversely, spore counts do not increase until much later in the storage period (Langston et al., 1958).

The usual conclusion derived from spore count data has been that the obligate anaerobes become vigorous late in the storage period if the pH of the material remains high (Barnett, 1954). However, it is evident that the high pH associated with spoiled silage was only the end result of the vigorous multiplication of vegetative cells of this organism in the period immediately after ensiling and the subsequent loss of lactic acid. The actual cause of the increase in lactate-fermenting bacteria will only be found by simultaneous studies of the physical, chemical, and bacterial changes which occur in the first few hr of the fermentation.

Unfortunately, the nature of the factors causing spoilage was not determined in this experiment although extensive environmental data were collected. Since it has been shown that the essential bacterial and chemical changes which occur in full-sized silos correspond to the results obtained with experimental silos, further work should be done with small silos which can be rigorously controlled.

SUMMARY

A study was made of the bacterial and biochemical changes occurring during the fermentation of forage-crop silages prepared under field conditions in farm sized silos.

As expected, well preserved silages were characterized by the formation of large amounts of lactic acid, small amounts of volatile base, and low pH values. In spoiled silages, the lactic acid which accumulated in the early stages of the fermentation was replaced by butyric and propionic acids. The formation of butyric acid was always associated with higher pH values, and was usually accompanied by the excessive production of volatile base.

Spoiled silages contained the same number of lactic acid bacteria as well preserved silages at every stage of the fermentation. The production of butyric acid in spoiled silages was associated with lactate-fermenting clostridia. These sporeforming anaerobes were present in large enough numbers to be isolated in the vegetative state with a nonselective medium.

REFERENCES

Woodman, H. E. 1925. Critical examination of the methods employed in silage analysis, with observations on some special chemical characteristics of "sour" silage. J. Agr. Sci., 15, 343-357.