EVALUATION OF SILAGE BY ORGANIC ACID DETERMINATION

by

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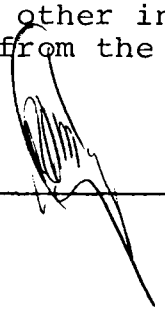
SIGNED: 

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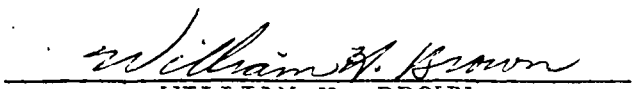

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ABSTRACT

An experiment was conducted in order to determine what differences occurred in both the organic acids and the proximate analysis of silage as affected by position in the silo and type of covering used. Organic acids, protein, ether extract, fiber, and lignin were determined in corn silage from three different areas in twenty different silos, both covered and uncovered.

It was found that transportation of the samples to the laboratory over dry ice and then freezing them until analysis was an acceptable means of preservation. Lactic and succinic acids were the only organic acids detected in the experiment with lactic being predominant.

It was further shown that a much higher concentration of lactic acid and ether extract was found in the middle and bottom sections of the silo than in the top section. The reverse was true for protein, fiber, and lignin where the top section of the silo contained a higher concentration than did either the middle or bottom sections.

Proper covering of the silo was shown to be a necessity if the nutrients in the top layer of the silo were to be preserved. The lower sections were not affected by covering.

Further evidence was deemed necessary before a complete evaluation can be made concerning nutritive value, digestibility, optimum intake, etc. of silage.

INTRODUCTION

A knowledge of the most appropriate criteria for evaluating the nutritive value of silage is necessary because of the popularity of this method of conservation of forages. It is well known that a major consideration in silage production is to achieve within the ensiled mass a sufficient concentration of lactic acid through rapid bacterial fermentation to inhibit other forms of microbial activity and thus preserve the product (2). Lactic acid is produced during the fermentation of green crops from carbohydrate present in the material and is normally the major acid produced. Acetic acid is the major volatile fatty acid produced. Various kinds and quantities of other organic acids accumulate in living plants and are destroyed in varying degrees during the ensiling process. Patterns of these acids in the living plant often help to characterize and differentiate types and modes of metabolism in plants. The same could well be true for these fermentation acids, lactic and the volatile fatty acids, in ensilage. Until the advent of gas-liquid chromatography, analysis for these various organic acids had been slow and inaccurate. By forming the methyl derivatives of these acids relatively accurate analyses can be accomplished by this improved method.

The major objective of this study was to determine what differences occurred in both the organic acids and the proximate analysis of silage as affected by position in the silo and type of covering used.

REVIEW OF LITERATURE

One major contribution to dairy feeding in recent times has been grass silage. Ingham (15) presented a brief history of silage making in America in which he stated that the modern method of ensiling grass silage started here in 1914. By the 1930's the dairymen realized the benefits of grass silage and began to use it. The crop most commonly ensiled was corn and was considered "The King of the Silo," not only because it produced heavy yields, but also because it was a cheap, high energy feed. In the process of fermentation in the silo, the natural sugars of corn were metabolized into organic acids that preserve the other nutrients. Of those acids lactic and acetic were the more important. Although high in sugar and starch, corn silage was low in protein, carotene, calcium, and phosphorus compared to alfalfa as a silage crop.

There are several factors that affect the nutritive value of corn silage. Owen (18) in a symposium paper on factors affecting nutritive value of corn and sorghum silage reported that heavy fertilization of corn generally improved the nitrogen content of resulting forage, but otherwise had little effect on nutritional quality. High density seeding had a detrimental effect on quality. Early-maturing or high-grain varieties and hybrids were usually equal or

slightly superior to late-maturing silages of low-grain content. Intake and milk yields were usually greater when silage was harvested in the ripe-seed stages of maturity, compared to early stages, even though efficiency of utilization was reduced. Seed content itself was clearly a poor indicator of quality. Conventional fine chopping and ensiling of the entire plant was as good as other tested methods of harvesting and processing. Urea could be safely used as a silage additive, but its value in silage relative to the common protein meals had not been satisfactorily established. Supplementation of high-silage rations with adequate protein, though difficult in practice, was important to efficient use of corn silage energy and protein.

Owens, Jorgensen, and Voolker (19) reported that when corn silage of high dry matter was compared with medium dry matter silage it produced lower total dry-matter yields, greater field losses, a higher percentage of ear losses, a lower carotene content, a lower total acid concentration, and a higher pH during fermentation. When fed to dairy cows dry matter consumption, milk production, and body weight gain were greater for cows fed the high dry matter corn silage. There were no significant differences in per cent milk fat or solids-not-fat between treatments. No consistent difference in chemical composition of the silages between years was found. Colovos et al. (9) found similar results when comparing nutritive value of silage for

cattle and sheep. Lopez et al. (17) reported similar results in respect to the concentration of organic acids related to dry matter content of the silage. In this study the corn was harvested at three different stages of maturity. Urea and soybean meal were added to the silage. The results showed that total organic acid concentration declined from 11.94% of dry matter in low dry matter samples after 42 days of fermentation to 3.14% in high dry matter samples. The concentration of the organic acids increased with storage time and was higher in the urea treated samples. Contrary to what was observed with lactic acid, volatile fatty acids were higher with the lower nitrogen supplementation. Acetic acid comprised 90% of the total volatile fatty acids.

Coppock (10) in a review of several reports about all corn silage feeding concluded that with one exception, specific nutritional problems associated with this kind of feeding have not been reported. The primary problem associated with feeding all corn silage lies not in the composition of the forage but in the limited knowledge concerning the type, most effective route, and economic level of supplementation necessary to meet the needs of dairy cattle. This is in agreement with the work of Hemken and Vandersall (14).

Colovas et al. (9) and Perry et al. (20) all studied the effect of stage maturity on nutritive value of

corn silage. They concluded that digestibility and nutritive value as well as intake were highest for silage harvested at the medium-hard dough stage of ear development.

Although some reports do not present satisfactory results for all corn silage programs, most of them agree in the feasibility of an all-corn silage program. As a matter of fact, Thomas, Brown, and Emery (26) affirm that cows can be fed corn silage as the only roughage for at least three lactations and maintain normal levels of milk production, reproduction, and animal health.

Organic acids in silage have been determined by several different methods and using different apparatus. Wiseman and Irvin (28) worked with celite columns with an internal indicator for the quantitative separation of silage acids ranging from butyric to succinic. The aqueous internal phase employed minimal amounts of sulfuric acid to prevent retention of the organic acids; sugar was added to the internal phase to increase elution resistance. Aqueous samples, 2 ml or less, were added directly to a dry column cap. Eluting solvents were mixtures of acetone with Skellysolve B. The method was designed to eliminate steam distillation, or ether extraction of acids, and mechanical equipment for collection of fractions. Single zone collections made possible a reduced number of titrations with increased accuracy. The column permitted fairly wide

separations of lactic and succinic acids, often difficult on silicic acid columns.

Later in 1961 Lessard, Briggs, and Scaletti (16) developed a method for the determination of volatile fatty acids and other silage acids such as succinic and lactic by gas chromatography. The method extracted organic acids from silage and separated them in the esterified form. The method was applied to a study of the volatile fatty acids and of lactic and succinic acids in alfalfa silage. The method showed that fresh alfalfa contains small amounts of lactic, acetic, and succinic acid. The quantities of these acids increased during fermentation of the silage but succinic and lactic acids decreased at later stages of fermentation, with butyric acid becoming prevalent. Wilting of the alfalfa crop for eight hours prior to ensiling decreased the rate of fermentation of all acids but prevented the later decrease of lactic and succinic acids. Butyric and propionic acids were present in only small quantities. A more severe wilting treatment decreased the rate of fermentation of acids still further. Several acids not detected by other methods were found in trace amounts.

In recent times several studies reported the determination of organic acids in silage by gas-liquid chromatography. Baumgardt (4) in 1964 stated that interest in gas-liquid chromatography was growing rapidly because of the extreme sensitivity, resolution and speed of analysis

possible with this analytical system. Application to the separation and quantitative analysis of volatile fatty acids in rumen fluid, blood and silage was of special interest to rumen nutritionists. Considerable time and effort were often spent in developing suitable operating conditions applicable to these biological fluids. The same author also reported some practical observations or suggestions during the operation of the equipment such as: the injector septums should be changed at least every four hours during continuous use, the system must be flushed with repeated injections of 10 ml of water and the injections system should be removed and cleaned periodically. A deposit builds up in the area of syringe needle injections which may become so dense that it will require the use of cleaning solution followed by thorough washing with water. The use of small injections and correspondingly high attenuation minimizes build up on the apparatus. Rumsey et al. (22) stated that the resolution of esters of some tricarboxylic acid cycle acids and other related organic acids by gas-liquid chromatography, present some difficulties in their isolation and separation. Keto acids were found to be difficult to extract and methylate because of their instability, while others decomposed when chromatographed. This author presented a procedure for the qualitative analysis of the methyl esters of some organic acids and for the preparation of forage and rumen fluid samples for

gas-liquid chromatography analysis. With this procedure the methyl ester peaks were tentatively identified by comparing the peak retention times of known single esters with a mixture of esters. The nine foot, 15% DEGS column completely separated the methyl esters of pyruvic, lactic, glyoxylic, oxalic, malonic, fumaric and succinic acids isothermally at 135°C in 13 minutes. The author affirmed that the technique of gas-liquid chromatography provides a relatively rapid method of analyzing for the organic acids mentioned above and could become a useful tool for studying the complex roles of the tricarboxylic acid cycle and other organic acids in intermediary metabolism in both plants and animals.

Rumsey et al. (23) reported a method to measure lactic, oxalic, molonic, fumaric, succinic, malic, alpha-ketoglutaric, and citric acids in forage, silage and ruminal fluids by gas-liquid chromatography. In silage samples lactic acid was considered of particular importance because it is a laboratory parameter for estimating silage quality. Recoveries of lactic acid indicated that it was recovered completely when added to silage extracts and that recoveries were unchanged due to the level of standard lactate added. Recovery results, coupled with the absence of difficulties when the samples were chromatographed, indicated that the gas-liquid chromatography method could be used to measure lactic acid in silage. This method was compared with the

$FeCl_3$ and p-hydroxydiphenyl method. The methods compared favorably for all samples except two. These two silage samples were of low quality and when analyzed with the $FeCl_3$ method did not maintain their normal color. Although it was difficult to determine which method was the most accurate, the chromatography method appeared to be equally applicable Succinic acid was the only other acid found in the silage analyzed. Although other carboxylic acids may have been present in silage samples, they were not detected by this method.

Clark (8) reported that trans-aconitic acid was the predominant organic acid formed in the leaves of the corn plant and all cereal crops studied. Malonic acid was detected in only small amounts in the corn plant while relatively high amounts of Malic acid were found in corn leaves. The other non-volatile Krebs cycle acids, including citric, succinic, fumaric, and isocitric acids were also found in the leaves of the corn plant. The author found different concentrations of organic acids between the cereal crops and the dycotyledonous plants.

The preparation of methyl derivates of some organic acids for analysis by gas-liquid chromatography was reported by Atkins and Canvin (1). They put one microgram to one milligram of the organic acid into two milliliters of dry, redistilled methanol with an excess of ethereal diazomethane for up to five hours. The solvents and excess reagent were

removed in vacuo at room temperature and the products recovered quantitatively in methanol. Samples of this solution were injected directly into the gas chromatograph. Methyl esters were also generated using methonolthionyl chloride. The retention time for the methyl ester of lactic was about three minutes and for succinic was approximately nine minutes. Separation by this method was not complete, especially for the more volatile derivatives, but this could probably be improved by using a longer column.

Candlish, with associates (6, 7), studied changes in levels of organic acids in corn silage treated prior to ensiling with formic acid, propionic acid, "chemstor," and "hay savor." The added acids were readily detected by gas chromatography. The addition of formic acid lowered the pH of the corn silage to a greater extent than the other added acids. The control silages contained between 5.81 and 17.43 mmols total acids per 100 ml of silage juice. Silage treated with propionic acid and "chemstor" contained between 33.86 and 42.53 mmol total acid per 100 ml silage juice. Formic acid treated silage contained intermediate amounts of organic acids. The acid treatment did not severely affect lactic acid production. The percentage of lactic acid in the organic acid fraction of silage juices dropped when other acids were added, but this drop was mainly due to the presence of the added acids rather than a decrease in lactic acid production. Lactic acid production increased when

0.15% formic acid or 0.75% "hay savor" was added to the silage; at higher levels of treatment, production decreased. Davidson, Stevenson, and Buchanan-Smith (11) following similar objectives but using alfalfa silage found that in untreated silage the pH dropped to 4.3 with high lactic acid production, but after 39 days, the pH began to rise as lactic acid was degraded by clostridia. Formic acid added at either the 0.33% or 0.50% level delayed but did not prevent either lactic acid production or subsequent degradation. Formic acid at 0.66% and all rates of formalin depressed lactic acid production. The production of butyric, isobutyric, and isovaleric acid were depressed to low levels only at the 0.66% rate of treatments. Formic acid was more effective than formalin in depressing volatile fatty acids.

Rumsey and Noller (21) studied various factors in the quantitative measurement of metabolic organic acid by gas-liquid chromatography. The conditions were established for quantitatively converting lactic, oxalic, malonic, fumaric, malic, alpha-ketoglutaric, and citric acids to their methylesters using methanol and concentrated hydrochloric acid; the esters were measured by gas-liquid chromatography using a DEGS column. Not all of the esters gave a linear response with increasing concentration levels, and the non-linearity appeared to be an effect of the GLC system. Lyophilization was suitable for dehydrating aqueous

samples. This method of drying represents a convenient means of adapting the gas-liquid chromatography method to the analysis of biological samples or aqueous extracts.

EXPERIMENTAL PROCEDURE

Three silage samples were collected from each of 20 different pit silos containing corn silage near Phoenix, Arizona. Samples were taken from three locations in the silo: (a) within the top six inches, (b) the middle section, and (c) two feet from the bottom. Immediately upon collection the samples were placed in plastic bags, labeled, and stored in an insulated ice chest over dry ice for transportation to the laboratory in Tucson the same day. The samples were then frozen until they were processed for analysis by gas-liquid chromatography, usually within one or two days. An additional three sets of samples from the same area of a single silo were transported to the laboratory over dry ice and then consequently treated differently before analysis: (a) processed immediately without further refrigeration the same day, (b) refrigerated overnight and then processed, and (c) frozen overnight and then processed. The purpose of this manipulation was to determine the effect of mode-of-storage on silage samples.

Gas-liquid chromatography (3, 5, 13) was used in determining the organic acids in silage and the samples were prepared by the method of Rumsey et al. (23), which included the following:

Distilled water (268 ml) was added to 32 g of wet silage and mixed in a Waring blender for ca 30 sec. The mixer bowl and contents were then cooled in the refrigerator for 30 min and again mixed for ca 30 sec. This process was repeated once after which the silage mixture was filtered into a 500 ml suction flask using a Buchner funnel. A 10 ml aliquot of the filtrate was then lypholized.

To the dry residue was added 10 ml of methanol and 0.5 ml of HCl. The mixture was shaken in a water bath for 4 hr at 55°C, cooled in a refrigerator and centrifuged to remove any solid material. The samples were then stored in a refrigerator until analyzed.

An aliquot of 5 µl of the methyl ester solution was injected into a Micro Tek Model No. DDS 167 gas chromatograph equipped with a 15% DEGS column (2 m by 6.3 mm) and a dual flame ionization detector. The esters were identified by comparison with retention times of authentic compounds and their relative amounts determined by an Infotronics Model No. CRS-108 printing integrator. Concentrations were determined by comparing response to that of standard solutions.

Oven dried (65°C) aliquots of the silage samples were analyzed for per cent ether extract and fiber (27) and lignin (12). Per cent protein was determined by the standard Kjeldahl method.

The data were analyzed by standard statistical methods (24, 25) and comparisons were made utilizing the "t" test to determine differences between the three different areas sampled in the silo.

RESULTS AND DISCUSSION

Table 1 shows the results from repeated injections of four different samples. This procedure was done to check the accuracy and repeatability of the technique. Only the fourth injection from sample D showed excessive variation from the mean and except for it the standard deviations from the mean are within acceptable error when working with biological materials. Thus it can be concluded that the method is sufficiently precise for this study as there was considerably less variation between injections from the same sample than there was among samples.

Table 1. Average Concentration of Lactic Acid and Standard Deviations of Four Injections from Four Different Samples

Sample	Injection No. 1	2	3	4	Mean	Standard Deviation
	(mmol/100 ml)					
A	2.032	1.965	1.626	1.761	1.846	0.184
B	1.422	1.219	1.287	1.355	1.320	0.149
C	1.490	1.490	1.558	1.693	1.558	0.094
D	0.984	1.084	0.880	1.355	1.066	0.200

Analysis of the three samples that were stored differently showed that the sample processed immediately contained slightly but not significantly, more lactic acid than the other two, there being 4.36, 3.99, and 3.92 mmol of lactic acid per 100 ml of silage in the immediately processed sample, the refrigerated sample, and the frozen sample respectively. On the basis of this analysis all silage samples were frozen upon returning to the laboratory until analyzed, usually within one or two days.

The concentration of lactic acid found in the different levels in each silo is shown in Table 2. Considerable variation was found both among silos and among levels within individual silos. Because the method used in analysis could not accurately detect values lower than 0.271 mmol/100 ml this value was assigned to all samples for statistical analysis where only trace amounts of lactic acid could be detected. Those values are reported as "trace" in the tables. It was shown that lactic acid concentration varied from trace amounts to 2.100, from 0.609 to 7.568 and from trace amounts to 5.826 mmol/100 ml in the top, middle, and bottom layers of the silo respectively. The average values for the top, middle, and the bottom layers were 0.60, 3.43, and 2.44 mmol/100 ml respectively. The top contained significantly less ($P < 0.01$) lactic acid than did either the middle or bottom layer. While the middle layer contained more lactic acid

Table 2. Concentration of Lactic Acid in Silage

Silo No.	Location of Sample in Silo			Visual Observations[a]
	Top	Middle	Bottom	
	-------(mmol/100ml)-------			
1	Trace	2.778	1.490	Well packed
2	2.100	3.116	2.100	Covered with plastic
3	1.219	1.355	1.287	Covered with grass
4	Trace	2.845	2.235	Well packed
5	0.542	4.200	4.268	Covered with plastic
6	Trace	4.200	2.439	Very well packed
7	Trace	2.439	1.152	Very well packed
8	Trace	2.032	0.745	Not well packed
9	Trace	4.471	5.149	
10	Trace	1.490	0.880	
11	Trace	6.233	5.826	Very well packed
12	Trace	7.588	0.339	
13	Trace	5.962	5.758	Very well packed
14	Trace	5.420	3.997	Very well packed
15	Trace	7.046	5.284	Not very well packed
16	Trace	0.677	Trace	Not well packed
17	Trace	0.609	Trace	Not well packed
18	Trace	2.845	1.626	Well packed
19	1.269	2.981	3.387	Covered with plastic
Average	0.60[b]	3.43[c]	2.44[c]	

[a]Unless otherwise stated the silo was not covered.

[b,c]The average values with different superscripts are significantly different ($P < 0.01$).

than the bottom layer they were not significantly different ($P > 0.05$). There were higher concentrations of lactic acid detected in the middle of those silos which appeared to be very well packed (visual observations in Table 2). All uncovered silos contained only trace amounts of lactic acid in the top layer. There was significantly more ($P < 0.01$) lactic acid in the top layer of the covered than uncovered silos (Table 3). This difference was not maintained ($P > 0.05$) in the middle and bottom layers, where no significant differences ($P > 0.05$) were noted between covered and uncovered silos.

Table 3. Average Concentration of Lactic Acid in Covered and Uncovered Silos

	Location of Sample in Silo		
	Top	Middle	Bottom
	---------- (mmol/100 ml) ----------		
Covered	1.27[a]	2.91[a]	2.76[a]
Uncovered	0.27[b]	3.33[a]	2.60[a]

[a,b]Values with different superscripts are significantly different ($P < .01$).

It is apparent from the foregoing data that a considerable loss of lactic acid takes place in the top layer of the silos. Much of this loss can be prevented by covering the silo with some suitable substance such as plastic. Proper packing is also mandatory if maximum lactic acid production is to occur as lesser amounts of lactic acid were detected in those silos which appeared not well packed. Condition of the ensiling material is also of importance as Lopez et al. (17) have shown that silage with a higher dry matter contained less organic acids than that with lower dry matter.

Succinic acid was the only organic acid other than lactic acid which was detected in the silage samples from this experiment. This is in agreement with the report of Rumsey et al. (23) who reported finding only these two acids. Any other organic acids which might have been present were probably destroyed during the ensiling process as it has been demonstrated that the technique of Rumsey et al. (23) which was used in this study will detect several other organic acids.

The level of succinic acid found in the silage sample from this experiment was quite low. Most samples contained only a trace with the highest level being only 0.015 mmol/100 ml of silage. Because of this low level the data did not warrant a table. Candlish and McKindy (7)

reported similarly low levels of succinic acid in untreated corn silage.

The result of the analysis of the silage samples for protein and ether extract are given in Table 4. Protein content was significantly higher ($P < 0.01$) in the top layer than in either the middle or bottom layers which were not significantly different ($P > 0.05$). There are several explanations available for this phenomena, the most logical being that during bacterial breakdown and mold formation on the top of the silo some protein was actually being produced. Obviously there was also a general loss of many fractions of the corn which would account for a concentration of protein provided it was not also broken down and lost. It is apparent that more research needs to be done in this area in order to accurately determine the fate of protein in the top of silos where there is an obvious substantial loss of total available nutrients.

The figures for protein found in the middle and bottom of the silo are an obviously more reliable measurement of the true protein content of the silage than are the figures from the top. While there was very little variation between the middle and bottom sections of the silo there was considerable variation among silos, there being a range of 5.11 to 9.29% protein. This large variation indicates a very large economic difference in the value of the product. While this variation was undoubtedly due primarily to

Table 4. Concentration of Protein and Ether Extract in Corn Silage from Different Locations in the Silo (Dry Weight Basis)

	Protein			Ether Extract		
	Location in Silo					
Sample	Top	Middle	Bottom	Top	Middle	Bottom
	(%)					
1	6.50	6.17	5.57	1.11	4.08	2.69
2	5.70	6.42	5.83	3.35	3.77	3.38
3	16.26	5.23	5.11	2.48	4.87	3.29
5	14.75	6.78	6.30	2.67	2.69	3.53
6	11.16	7.53	7.54	1.77	2.90	2.79
7	9.69	7.78	6.71	1.09	3.88	3.51
8	7.84	8.60	6.89	0.82	1.70	1.40
11	11.05	7.67	6.32	1.52	1.62	2.50
13	10.64	7.61	6.15	0.67	1.85	2.26
14	10.19	7.69	7.54	0.99	1.60	1.92
15	10.99	7.81	6.65	0.64	2.05	1.58
16	7.29	6.88	6.88	1.57	1.91	1.45
17	10.97	9.29	8.64	1.64	1.96	1.81
20	8.30	8.65	9.18	1.70	2.08	1.60

variation in the material that was ensiled, method of ensiling may have exerted some influence.

It was shown that a highly significant loss ($P < 0.01$) of the ether extract fraction of silage occurred in the top layer of the silos. There was no significant difference ($P > 0.05$) between the middle and bottom layers. The loss of ether extract from the top would be expected in view of the loss found in lactic acid. The high loss indicated for ether extract in the top of the silo would represent a significant economic loss especially when the high energy content of fat is considered.

Fiber content of silage varied from 25.68 to 41.95% in the bottom and middle layers of the silos (Table 5). Even though the top layer had a higher average fiber content than the other two layers this difference was not significant ($P > 0.05$). It becomes increasingly evident as each successive parameter of this study is examined that many factors influence the nutrient content of a particular silage. No attempt was made in this study to examine the material going into the silo as it was processed. It is evident that before excessive conclusions can be drawn regarding particular silage in a silo evidence regarding the condition of the ensiling material and method of handling must be thoroughly examined.

Lignin content of silage was examined from only six silos (Table 5). Even on this small number a very high

Table 5. Concentration of Fiber and Lignin in Corn Silage from Different Locations in the Silo (Dry Weight Basis)

	Fiber			Lignin		
	Location in Silo					
Sample	Top	Middle	Bottom	Top	Middle	Bottom
	(%)					
1	38.87	38.39	30.23	4.43	4.61	4.13
2	42.10	35.64	37.52	3.99	3.74	3.91
3	42.11	40.98	41.95	11.31	11.92	11.80
5	44.04	36.77	40.55	9.82	8.28	10.86
6	49.03	38.88	40.47	11.52	4.99	5.46
7	45.49	39.98	39.80		5.54	6.19
8	36.40	28.71	28.04			
11	32.18	30.46	32.51			
13	32.12	28.94	28.18			
14	31.93	26.58	25.68			
15	33.20	26.80	31.26			
16	28.06	28.48	28.23			
17	30.18	30.63	30.89			
20	28.62	27.19	27.56			

variation among silos was evident, there being a range of 3.74 to 11.92% lignin. Conclusions based on so small a number are risky at best but one bit of evidence seems obvious in that the top section of the two uncovered silos (Nos. 6 and 7) may have suffered a 50% loss of nutrients. This being based on the fact that the lignin content of the top was approximately twice as high as the middle sections. Lignin being quite inert, could only increase in concentration when other material was removed. Again there is overwhelming evidence that factors such as stage of maturity, packing of the silo, whether the silo was covered, etc., greatly influence the chemical content of the resulting silage (Table 6). Further evidence is mandatory before conclusions can be drawn regarding the nutritive value, digestibility, optimum intake, etc., of these silages.

Table 6. Average Concentration of Protein, Ether Extract, Fiber, and Lignin in Corn Silage from Different Locations in the Silo (Dry Weight Basis)

	Location in Silo		
	Top	Middle	Bottom
	----------(%)----------		
Protein	10.09[a]	7.44[b]	6.80[b]
Ether Extract	1.57[a]	2.71[b]	2.41[b]
Fiber	36.73[a]	32.69[a]	33.06[a]
Lignin	7.80	6.03	6.02

$t_{0.01} = 2.78$.

[a,b]Values with different superscripts in the same line are significantly different ($P < 0.01$).

CONCLUSIONS

Organic acids, protein, ether extract, fiber, and lignin were determined in corn silage from three different areas in twenty different silos, both covered and uncovered. The following conclusions were drawn:

1. Transportation of the samples to the laboratory over dry ice and then freezing them until analysis was shown to be an acceptable means of preservation.
2. Only two organic acids were detected; lactic and succinic, with lactic being predominant.
3. A much higher concentration of lactic acid and ether extract was found in the middle and bottom sections of the silo than in the top section.
4. A lower concentration of protein and fiber and lignin was found in the middle and bottom sections of the silo than in the top.
5. Proper covering of the silo was shown to be a necessity if the nutrients in the top layer of the silo were to be preserved. The lower sections were not affected by covering.
6. Further evidence is necessary before a complete evaluation can be made concerning nutritive value, digestibility, optimum intake, etc. of silage.

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