STORAGE TRIALS WITH WET BREWERS' GRAINS

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ABSTRACT

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Studies were conducted during the summer to determine how best to store and preserve wet brewers' grains. Several methods of preservation were studied. Thirty pounds of wet brewers' grains were stored for 32 and 76 days in plastic buckets. Complete preservation was achieved by sealing with plastic foam. Mixing the grain with yeast (10%) decreased spoilage. There was formation of acetic, propionic and butyric acid and changes in the protein fraction. In a second experiment, 300 pounds of wet brewers' grains were stored for 32 and 60 days in steel barrels. Covering the grains with a plastic bag filled with water or adding 2% propionic acid or 1.4% formic acid plus 0.1% paraformaldehyde resulted in complete preservation. Ethanol and lactic acid were the main fermentation products. Wet brewers' grains can be stored successfully under anaerobic conditions, by adding propionic acid or by adding formic acid and paraformaldehyde.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

By-products from the processing of various plant materials used in the manufacture of products for human consumption can be used for livestock feeds. In this practice these products are not wasted but rather transformed to a superior kind of food suitable for human consumption. Brewers' grains are a product of the beer industry that can be incorporated in significant proportions in the diets of animals, particularly ruminants. The production of this grain is greatest in spring and summer, when pastures are green, and least in fall and winter when demand for feed is great. Consequently, marketing wet brewers' grains as livestock feed presents a problem, especially during summer. Wet brewers' grains can be dried to about 10% moisture which assures a stable, storable product. However, this drying process requires considerable energy because of the high water content. Also, there is a pollution problem caused by dust, odor, smoke, etc. On the other hand, the fresh product sold now by the breweries spoils in less than a week if not stored properly. Spoiled grains can cause serious health problems when fed to animals. A practical way to store wet brewers' grains on the farm needs to be found.

The objectives of this research were: (1) to study methods of storage of wet brewers' grains using small model silos, and (2) to describe the chemical and nutritional changes that occur during storage.

LITERATURE REVIEW

The National Academy of Sciences (1971) defined brewers' grains as the coarse, insoluble residue from brewed malt, and classified them as protein supplements. During 1973 there were 138,445,000 barrels of beer produced in the United States (World Beer Production: 1971-1974). Brewers dried grains production during the same year was 348,000 tons (U.S.D.A. Agricultural Statistics, 1975). This figure does not account for brewers' grains sold on a wet basis, estimated at 37% of the total production (Hunt, 1969). Therefore, the total production of brewers' grains on a dry basis was about 552,000 tons.

Brewers' grains result from a process that involves solubilization and isolation of part of the starch from barley. Barley contains little or no amylase in the ripe seed. Thus, for making beer the cereal is allowed to germinate to synthesize enzymes and then dried and stored until needed. Such germinated dried barley is known as malt. During germination the starch in the malt is only slightly hydrolysed since it is physically protected from amylase action by the cellular structure in the seed. Accordingly, the first step in brewing is the grinding of the malt and its suspension in water so as to permit hydrolysis of the starch. After saccharification

has reached the desired stage the mixture is boiled to stop further enzymatic action, then filtered. The solid wet filtrate is called brewers' grains (Stainer, 1970). Hops are added to the malt liquor to give beer a bitter flavor and later filtered. The filtered hops are mixed with the brewers' grains in a proportion of about 3% following pressing the wet material to reduce moisture. The grains can be dried in a rotatory oven to about 90% dry matter. This product is stable.

The composition of brewers' grains compared with corn silage, corn and soybean meal is listed in Table 1. Brewers' grains are relatively high in protein, they have about three times as much digestible protein as corn, but a much lower energy content. Digestible and metabolizable energy values are comparable to those in corn silage. Potassium content is very low. This low potassium level results from the high solubility of potassium salts in the malt and they remain in the filtrate. Barley contains 0.52% potassium, wort sediment 0.90%, yeast 1.96% and beer 0.62%, on dry matter basis (Pomeranz and Dikeman, 1976).

Nitrogen free extract in brewers' grains consists mainly of pentosans. They make up 25.2% of the dry matter since most of the starch from the barley grain has been hydrolyzed to glucose and removed in the brew liquid (National Academy of Sciences, 1971).

Table 1. Nutrient Composition of Brewers' Grains, Corn Silage, Corn and Soybean Meal (% DM).[a]

	Brewers' Grains	Corn Silage	Corn	Soybean Meal
Ref. No.	5-02-141	3-02-822	4-02-879	5-04-600
Ash	4.2	5.7	1.6	6.7
Crude fiber	16.1	21.6	2.4	6.8
Ether extract	7.2	3.1	4.7	5.2
N-free extract	44.2	58.5	80.3	34.6
Protein	28.3	8.2	10.9	46.7
Dig. protein (Cattle)	21.0	3.8	7.5	39.7
T D N (Cattle)	66.3	67.6	88.8	84.9
Calcium	0.30	0.50	0.05	0.31
Phosphorus	0.53	0.20	0.35	0.65
Sodium	0.28	----	0.34	0.27
Potassium	0.10	0.88	0.80	1.93
DE cattle Mcal/Kg	2.92	2.98	3.92	3.74
ME cattle Mcal/Kg	2.40	2.44	3.21	3.07

[a] National Academy of Sciences, 1971

Brewers' grains used as animal feed

Brewers' grains are used as feed for several species of animals. They are a good source of protein. The relatively high crude fiber (16%) is characteristic of roughages.

Poultry. Both dried and wet brewers' grains have been used successfully in poultry rations. Laying hens fed brewers' dried grains at levels of 5 and 10% of the diet did not have a significant difference in feed intake or in body weight gain or in the number or weight of eggs when compared to the hens consuming a commerical ration. The diets had 2500 to 2800 cal/g metabolizable energy and crude protein was 17 to 18% (Laurent and Vanssay, 1971). However, others have reported that addition of 10% brewers dried grains plus yeast to a corn-soybean meal diet resulted in an increase in egg weights and egg numbers; interior egg quality was also improved (Eldred et al., 1975). Levels of brewers' grains as high as 20% of the total ration have been suggested for laying hens (Couch, 1976).

An experiment was conducted with starters (0 to 8 weeks) and growers (8 to 18 weeks) of a commercial egg producing strain of chickens. For optimal performance the diet should not exceed 10% brewers dried grains for starters or 30% for growers. These experimental rations, with and without brewers' grains had about 22% protein and similar energy contents (Ademosun, 1973). For broilers a ration that was 32% wet Brewers' grains silage plus 10% molasses or 42% wet brewers' grains silage was tested. Weight at the end of

seven weeks was the same in the control and in the test groups (1600 g). The silage was preserved with propionic acid at the level of 2% (Wegner, 1973).

Swine. In contrast to poultry, no studies using wet brewers' grains in swine feed were found.

When 15% brewers dried grains was included in the pre-starting diet for pigs until they reached 15 kg, and then 20% until their weight was 95 kg, satisfactory results in weight gain and carcass quality were obtained (Branckaert and Vallerant, 1972). Young and Ingram (1968) conducted an experiment in which brewers dried grains furnished 0, 25, 50, 75 or 100% of the supplemental protein in a corn-soybean meal diet for growing-fattening pigs. They found no difference in growth rate or carcass quality up to 50% of the supplemental protein. The digestible energy was 52.3% for brewers dried grains alone and 55.8% for brewers dried grains plus 5% yeast. The estimated metabolizable energy was 2.38 and 2.50 kcal/g, respectively (Kornegay, 1973). For comparison digestible energy in corn for swine is 3.44 kcal/g and metabolizable energy 3.22 kcal/g (National Academy of Sciences, 1971). Reproductive performance of sows was very acceptable when either 20 or 40% of the diet was derived from brewers dried grains. The diets were readily consumed and palatability was not a problem. The rations with and without brewers' grains were formulated to have 15% protein and equal levels of lysine and metabolizable energy (Wahlstrom and Libal, 1976).

Beef and dairy. The major proportion of the brewers' grains produced is fed to beef and dairy cattle. Some farmers feed brewers' grains on a regular basis. The level may be as much as 20% of the diet (Bullock, 1974; Stephens, 1976). Increased nitrogen retention was reported when brewers dried grains plus 5% brewers dried yeast were added to a high urea semipurified diet for fattening steers (Hatch et al., 1972). The net energy value of brewers dried grains for beef cattle maintenance was determined to be 2.3 kcal/g and was 1.4 kcal/g for gain (Preston et al., 1973). The incidence of rumen parakeratosis and abcessed livers was low for beef cattle fed brewers dried grains when compared with other low roughage rations (Johnson, 1973; Preston, 1973). In other experiments dairy cow rations low in protein were supplemented with distillers dried grains, brewers dried grains or urea. Distillers dried grains and brewers dried grains gave similar effects on milk yield, milk fat, weight gain and feed intake. These rations were superior to the ratios that had urea or low protein (Loosli and Warner, 1968). Griffiths (1971) found that for cows in mid lactation milk production and composition were the same when a 18.5% crude protein concentrate was diluted 2:1 with brewers dried grains.

Wet brewers' grains silage has been fed successfully to cattle. In one trial with dairy cows 15 kg lucerne silage were replaced by 11.5 kg wet brewers' grains silage. Milk yield was not affected but the fat content was reduced and the iodine number of the fat increased. Average production

of the cows fed brewers' grains was 16.6 kg of 4% milk per cow daily (Axelsson and Hellberg, 1941). Studies have been conducted to compare milk production when brewers' grains silage or silage made from sugar beet tops was fed to cows. Milk production was the same, but the milk contained less dry matter and fat when brewers' grains silage was fed. However, no adverse effects were observed when this milk was fed to infants (Mollenbach and Larsen, 1953). Orth and Kordts (1965) conducted a study to determine the effect of feeding 10 kg of wet brewers' grains silage on milk quality. Taste, smell or bacterial counts of milk were not affected. However, butter was softer and the iodine number higher. There was no decrease in milk yield, milk fat or milk protein. Also, the cows were in good health.

The nutritive value of brewers' grains silage was reported by Hashimoto et al. (1971). Digestibility coefficients for dairy cows were 73% for crude protein, 28% for crude fiber and 67% for nitrogen free extract. Porter and Conrad (1971) compared the nutritive value of wet brewers' grains, brewers dried grains, distillers dried grains and solubles and a combination of wheat bran and soybean oil meal for milk production. These grains made up 20% of the concentrate mixture on a dry matter basis. Milk yields were the same for all concentrates. Cows ate less dry matter when wet brewers' grains were fed, but the digestibility was higher. A product called "Maltlage" that is marketed consists of 65% wet brewers' grains, 32.75% corn and 2.25% of a vitamin-mineral supplement. Rakes and

Davenport (1975) fed this product to lactating dairy cows at the level of 0, 40 or 50% of the total ration. The control diet was 71.6% corn silage, 6.5% soybean meal, 21% corn and 0.9% vitamin-mineral supplement. There were no differences in milk production.

There has been a considerable number of studies that demonstrate the utility of brewers dried grains in livestock feeding. Although less effort has been devoted to similar studies with wet brewers' grains silage, they are apparently equally useful.

Preservation of brewers' grains

Wet brewers' grains produced by the breweries have about 80% moisture. They can be dried to about 10% moisture. This product is stable, but the process of drying implies high energy cost, and current restrictions on atmospheric contaminants (dust, odor, smoke, etc.) result in large costs for capital, operation and maintenance. Equipment malfunction is an additional problem (Linton, 1973). To reduce the cost of drying, the grains are pressed to reduce their moisture content. The resulting press water or effluent containing both suspended and soluble solids, can present a serious disposal problem (Finley et al., 1976). Brewers' grains liquor have a biological oxygen demand (B.O.D.) of 22,500 milligrams per liter. This liquor may account for 30 to 60% of the B.O.D. and suspended solids generated by a brewery (Hang et al., 1975).

Fresh brewers' grains are a highly perishable product due to their high moisture content, nutrient composition and the microbial contamination to which they are exposed. When the grains cannot be fed to the animals within a period of a few days, they need to be stored. Bad storage conditions result in losses due to spoilage. Fritzch and Abadjieff (1967) attributed several cases of illness in cattle to moldy brewers' grains silage.

Several additives have been used to assure good preservation of wet forages stored in silos (Watson and Nash, 1960). In the case of brewers' grains several methods of storage and the use of different additives have been tested.

Wet brewers' grains ensiled with no additive had a dry matter loss of 17.5%. With three liters of a 2N AIV solution per 100 kg the dry matter loss was 11.6% and with five liters 6.4% at pH 2.3. The quality of the silage was similar in all cases but the loss of dry matter was reduced by rapid acidification (Krinstad and Ulvesli, 1951). Wet brewers' grains stored in a water proof concrete silo has 12.2% loss of organic matter. In an earth pit the losses were higher. The silo silage was of better quality than the silage from the earth pit and had less butyric acid content (Dijstra, 1955).

A positive correlation was established between dry matter loss and butyric acid concentration in wet brewers' grains ensiled in round concrete silos and kept from four to eight months (Schoch, 1956). Silage from brewers' grains, as evaluated by butyric acid content, was unsatisfactory without

additive or with 0.20 to 0.27% sodium chloride, 4.2 to 8.4% dried beet slices or 4 to 10% dried pear residue. Good results were obtained when 10 to 15% dried apple residue, 5 liters 1.1N formic acid or 5 liters of 2N AIV solution per 100 kg of grain were added. Draining off the juice from the start of the ensiling period reduced losses of dry matter. The composition of fresh brewers' grains and of the silages with or without additives was similar (Schoch, 1957).

Three and one half tons of wet brewers' grains were stored under anaerobic conditions in a wooden silo lined with polyethylene. Eleven pounds of sodium chloride were added per ton of grain. After three weeks 23 gallons of seepage had been gathered. Dry matter losses were 10.9% after 20 weeks. During storage the pH fell from 4.7 to 3.9, but in the spoiled material pH increased to 8.4. In order to reduce the losses still further, higher levels of salt and better sealing were recommended (Myers and Ollier, 1962).

The use of airtight silos has given successful results for the storage of wet brewers' grains mixed with supplemental feeds. In this case wet brewers' grains were pressed to reduce their moisture content to a 68-72 percent range. Addition of dry grain and a mineral-vitamin mixture reduced the moisture content further to 49-54% (Anonymus, 1969; Anonymus, 1976).

Using 200 ml test tubes as model silos, Allen and Stevenson (1975) showed that addition of 0.50 and 0.75% formic acid, and 0.75% of a formic-propionic acid mixture resulted in good quality silage.

Formic acid or propionic acid at 0.40% or a mixture of formic and propionic acid at 40% reduced spoilage in uncovered piles of wet brewers' grains. Depth of discoloration and spoilage after 14 days of storage was from 5 to 7.5 cm in the grain treated with the formic-propionic acid mixture and 23.5 cm in the untreated grain. Addition of 2% molasses did not have beneficial effects on the conservation of the grains (Allen et al., 1975).

In conclusion, storage and conservation of brewers' grains depends primarily on the characteristics of the silo where the grains are to be kept. Good results are to be expected under strict anaerobic conditions. In this way the growth of lactic acid forming bacteria is assured. The acidity produced by these bacteria will inhibit the growth of putrefactive molds. If the grains are not stored in airtight silos, the addition of preservatives must be considered. To enhance an active lactic acid fermentation that will inhibit other microbial growth, initial acidification of the mass and the addition of readily available carbohydrates for the lactic acid bacteria can be tested. It is possible that an increase of the lactic acid bacteria population by means of an inoculation could inhibit or stop the growth of other undesirable microorganisms. The silage obtained must not have lost the nutritional characteristics of fresh feed. During the ensiling process toxic substances must not be formed. Additives used must be innocuous when fed to the animal. The final product must be palatable and acceptable for the animal.

Additives must be easy to handle and harmless for the persons using them and for the storage structures. Furthermore, they must be economical.

MATERIALS AND METHODS

Two storage trials were conducted with wet brewers' grains and brewers' yeast obtained by truck from the Strohs Brewery in Detroit. Both trials were conducted during hot weather.

Trial 1

Fresh brewers' grains alone and grains mixed with yeast (10%) were placed in five gallon plastic buckets. Dimensions of the buckets were: bottom diameter 10 in., top diameter 13 in. and height 14 in.

A thermocouple was placed in the center of the mass to monitor temperature changes during the ensiling period. Samples of grain were taken on days 4, 8, 15 and 22 for pH, ethanol, volatile fatty acids (acetic, propionic and buytric), lactic acid and ammonia. These samples were taken from the unspoiled part of the grain. Samples of the original material and of the material ensiled for 32 and 76 days were taken for analysis of dry matter, protein, ammonia, acid detergent fiber, acid detergent insoluble nitrogen, ethanol, volatile fatty acids and lactic acid. These samples were taken after emptying the barrels from the part of the material that was not spoiled. Spoilage was separated by hand and its weight determined.

Four buckets were used for each treatment, two with wet brewers' grains alone and the other two with wet brewers' grains plus 10% autolized wet brewers' yeast. One of the buckets to which yeast was added and one of the buckets without yeast were removed and emptied to determine the amount of spoiled material after 32 days and the other two after 76 days.

The various chemicals were mixed with 60 lb of brewers' grains in a horizontal mixer and then 30 lb divided into each bucket. The exact weight of the content of each bucket was recorded.

Lactobacillus cultures were grown in sterilized milk autoclaved at 130°C for 30 minutes. Two hundred ml of this culture were mixed with 60 lb of grain.

The buckets were placed in a heated room, around 76°F, to simulate hot weather conditions.

Table 2 gives a description of the type of ensiling method and material used. A split-plot design with repeated measurement was used to analyse the results of this experiment. Seventeen different additives or ensiling methods and the presence or absence of yeast formed 34 treatment combinations. The periods were the days after the initiation of the experiment in which the data were collected. Each treatment combination had two repetitions. Bonferroni's "t" test was used to evaluate differences of group treatment means. Correlations were determined between the averages of the measurements or composition of 32 and 76 days silage.

Table 2. List of additives or method used to ensile wet brewers' grains. Trial 1[a]

Treatments		# buckets
1	None	4
2	Propionic acid (0.5%)	"
3	Propionic acid (1.0%)	"
4	Ammonium propionate (0.5%)	"
5	Ammonium isobutyrate (0.5%)	"
6	Ammonia (0.3% nitrogen)	"
7	Paraformaldehyde (0.1%)	"
8	Formic acid to pH 3.2 plus paraformaldehyde (0.1%)	"
9	Potassium carbonate (1.5%)	"
10	Foam sealants	"
11	Potassium carbonate (1.5%) plus propionic acid (0.5%)	"
12	Sulfuric acid to pH 3.6	"
13	Formic acid to pH 3.6	"
14	Dried molasses (3%)	"
15	Sucrose-starch mix (1:1) (3%)	"
16	Sodium benzoate (0.1%)	"
17	*Lactobacillus casei* plus *L. bulgaricus* culture	"
18	*Lactobacillus casei* culture in grain without yeast	2

[a] The numbers in parenthesis are the concentrations of the additive mixed with the wet grains, except where indicated.

Trial 2

Seventeen treatments with three replications each were used. The experimental units were 55 gallon steel barrels containing 300 lb of wet brewers' grains. The grain was mixed with the additives in a mixer, 300 lb at the time, and placed immediately in the barrels lined with plastic bags (38 x 65 in., .004 in. gauge). Two barrels of the three repetitions were emptied at the end of 32 days and samples collected. These samples, as well as the original fresh material were analysed for dry matter, total nitrogen, ammonia, acid detergent fiber, acid detergent insoluble nitrogen, ethanol, volatile fatty acids and lactic acid. Thermocouples were placed in the center of the mass of the barrel to monitor temperatures.

Samples of treatments 1, 2, 3, 4, 5, 6, 11, 13, 14, 15, 16 and 17 were placed in 100 ml test tubes at room temperature and frozen on days 5, 7, 10 and 13 after the initiation of the experiment. Analysis for volatile fatty acids, lactic acid, ethanol and ammonia were made on these samples. Spoiled material was separated by hand and samples from the unspoiled material were taken from different sections at the silo.

Calcium sulphate was added as a slurry to cover the grain. About 10 lb of limestone, dried molasses, liquid molasses or ground corn were used for treatments 7, 9, 10 and 12, respectively to form a seal about 1 cm thick on top of

the grain. A commercial product called "Super Silo-Zime"* was the lactic acid bacterial culture used, and 340 g were mixed with 300 lb of grain. This is the equivalent to the 5 lb per ton recommended by the manufacturer. The barrels were in a large building where air circulated freely. A split-plot design with repeated measurement was used for statistical analysis of the results of this experiment. The number of treatments was 17 and the periods were the days after the initiation of the experiment in which the data were collected. Each treatment had three replications. Bonferroni's "t" test was used to evaluate differences of treatment means. Correlations were determined between averages of measurements or composition of 32 and 60 days silage. (Table 3)

Analytical methods

Dry matter was determined by drying samples in an oven at 105°C overnight. Total nitrogen was determined using the Kjeldahl method (AOAC, 1965). Copper sulphate was used as the catalyst.

Acid detergent fiber and acid detergent insoluble nitrogen were determined using the Van Soest method (Goering and Van Soest, 1970). Samples for these analysis were dried for 48 hr at 45°C in an air forced oven. Samples of the grain were diluted 1 in 10 with water, homogenized and filtered through four layers of cheesecloth. pH values were taken from this filtered homogenate. After centrifugation at 27,000 g

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* Biochemical Corporation of America, Salem, VA 24153

Table 3. List of treatments used to ensile wet brewers' grains. Trial #2[a]

1	Control, no additive
2	Propionic acid (1%)
3	Propionic acid (2%)
4	Formic acid (1.4%)[b] plus paraformaldehyde (0.1%)
5	Sulfuric acid (0.3%)[c]
6	Butylated hydroxyanisole (B H A) (200 ppm)
7	Ground limestone on top of the grain
8	Calcium sulphate on top of the grain
9	Dried molasses on top of the grain
10	Liquid molasses on top of the grain
11	Liquid molasses (7%)
12	Ground corn on top of the grain
13	Ground corn (10%)
14	Lactic acid culture, 340 g/300 lb grain
15	Lactic acid culture, 340 g/300 lb grain plus liquid molasses (7%)
16	Lactic acid culture, 340 g/300 lb grain plus ground corn (10%)
17	Sealed with a plastic bag full of water on top

[a] The numbers in parenthesis represent the concentration of the additive mixed with the wet grains, except where indicated.

[b] 2200 ml 85% formic acid/300 lb grain

[c] 1000 ml 40% sulfuric acid/300 lb grain

for 15 min to precipitate the proteins. The supernatant was used for volatile fatty acids, ethanol and lactic acid determination.

Ammonia was determined by a colorimetric method used for blood ammonia (Okuda et al., 1965) and modified by Kulasek (1976). The method of Barker and Summerson (1941) was used for lactic acid analysis.

Volatile fatty acids (acetic, propionic and butyric) and ethanol were measured using a Hewlett-Packard gas liquid chromatograph model 5730A with flame ionization detector. A glass column (6 ft x 2 mm ID) was packed with 3% Carbowax 20 M, 0.5% H_3PO_4 on 60/80 Carbopack B (Supelco, Inc. 1-1825). Nitrogen was the carrier gas at a flow rate of 60 ml/min. The temperature program used was two minutes beginning at 140°C with a temperature increase of 4°C/min for ten minutes, and finally eight minutes at 180°C. Prior to the injection the samples were acidified with a drop of 9N H_2SO_4. Injection volume was 3 microliters. Concentrations were calculated relating the areas under the peaks for the standards with the areas under the peaks of samples.

Total microbial count on the grain was made using agar plates. The dilutions considered for plating were 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Plates were incubated at 32° for 48 hours. For coliform bacteria the medium used was violet red bile agar with plate dilutions of 10^{-2}, 10^{-3} and 10^{-4}, incubated at 37°C for 24 hours. Yeast and mold determinations were made using acidified potato dextrose agar, pH 3.5. The

plates were incubated at room temperature for five days for the dilutions of 10^{-2}, 10^{-3} and 10^{-4}. A lactobacillus broth (pH 5.4) was used for lactobacillus estimation with dilutions of 10^{-2}, 10^{-3} and 10^{-4} incubated at room temperature for three days.

RESULTS AND DISCUSSION

Trial 1

Four days after the beginning of the experiment most of the buckets had developed colonies of mold on the surface. After six days, flies started to grow beneath the surface of the grain of treatment no. 9 (potassium carbonate). Two days later grain in buckets treated with propionic acid (0.5%), ammonium isobutyrate and sodium benzoate started to spoil. After 32 and 76 days when the buckets were emptied, all the treatments but the one sealed with foam had the surface layer of the grain decomposed. Flies were observed growing in the spoiled part of the grain. Digging into the mass to take samples from the unspoiled part of the grain hastened spoilage.

Spoilage. All buckets except those sealed had considerable spoilage (Table 4). Eighteen percent of the grain was spoiled in one bucket covered with foam that had a leak between the foam and the edge of the bucket. However, the grain in the other three sealed buckets had no spoilage. This demonstrates that when anaerobic conditions are maintained spoilage can be prevented. Other treatments that reduced spoilage were propionic acid (0.5%), formic acid plus paraformaldehyde, sucrose-starch mix and bacterial culture. These averaged 24.3% spoilage compared to 30.5% for

Table 4. Percentage of spoiled material after ensiling wet brewers' grains. Trial 1[a]

Treatments	% spoiled after 32 days	% spoiled after 76 days	$\bar{x}$
Control	28.0	33.0	30.5
Propionic acid (0.5%)	24.0	28.0	26.0
Propionic acid (1.0%)	30.5	45.0	37.0
Ammonium propionate (0.5%)	33.0	43.5	38.3
Ammonium isobutyrate (0.5%)	25.0	34.5	29.8
NH_3 (0.3% N)	30.0	40.0	35.0
Paraformaldehyde (0.1%)	27.5	38.0	32.8
Formic acid to pH 3.2 + paraformaldehyde (0.1%)	17.0	31.5	24.3
K_2CO_3 (1.5%)	33.5	38.5	36.0
Foam sealants	00.0	9.0	4.5
K_2CO_3 (1.5%) + propionic acid (0.5%)	33.0	44.0	38.5
H_2SO_4 to pH 3.6	31.0	37.5	34.3
Formic acid to pH 3.6	23.0	41.5	32.3
Dried molasses (3%)	27.0	42.0	34.5

Table 4 (cont'd.)

Treatments	% spoiled after 32 days	% spoiled after 76 days	$\bar{x}$
Sucrose-starch (3%)	22.5	24.5	23.5
Sodium benzoate (0.1%)	25.5	38.0	31.8
Lactobacillus casei + L. bulgarious	19.0	28.0	23.5
Lactobacillus casei[b]	38.0	37.0	37.5
Wet brewers' grains without yeast[c]	27.6	36.4	32.0
Wet brewers' grains + 10% yeast[c]	23.5	33.8	28.9
Average	25.6	35.1	30.4

[a] average of two values

[b] one value

[c] average of 35 values

the control. Addition of yeast reduced spoilage from 32.0 to 28.9%. The unspoiled grain treated with propionic acid (1%) and sulfuric acid was darker than the unspoiled grain from all the other treatments. The odor of the unspoiled grain was objectionable in all, except the sealed buckets, due in part to the contact with the spoiled material.

pH. Fresh brewers' grain had a pH of 5.3. After the second day of ensiling pH had decreased to values close to 4.0 in all the treatments except in those containing potassium carbonate. Addition of ammonia did not prevent the usual decrease in pH (Table 5).

Analysis of variance for pH on days 2, 8, 15 and 22 indicated that effects of additives and days were highly significant. The effect of presence or absence of yeast was not significant. The interactions of additives, yeast and days, were significant (Table 6). Figure 1 shows that the pH increased in day 15 and decreased after day 22 due to the addition of yeast.

Comparison of treatments means from day 2 to 22 indicated the following: The pH of the acid treatments propionic (0.5 and 1.0%), formic acid, formic acid plus paraformaldehyde and sulfuric acid was not significantly different from the pH values of the control. Addition of potassium carbonate increased average pH from a control value of 4.03 to 5.06 ($P<.01$). The pH of the grain treated with propionic acid alone was lower than the pH of the grain treated with ammonium propionate or potassium carbonate plus propionic acid, but

Table 5. Changes in pH with time in wet brewers' grains ensiled with different additives. Trial 1[a]

Treatment	Day						$\bar{x}$
	2	8	15	22	32	76	
Control	4.18	3.98	3.90	3.80	3.70	4.60	4.03
Propionic acid 0.5%	4.08	3.84	3.72	3.72	3.72	4.40	3.91
Propionic acid 1.0%	4.28	4.52	4.91	4.70	3.82	3.90	4.36
Ammonium propionate 0.5%	4.74	4.93	4.98	4.89	4.10	4.42	4.68
Ammonium isobutyrate 0.5%	3.96	3.98	4.21	4.21	3.95	4.52	4.14
NH_3 0.3%	3.78	3.91	4.14	3.91	3.70	4.45	3.98
Paraformaldehyde 0.1%	4.15	3.94	4.61	3.85	3.72	4.78	4.18
Formic acid to pH 3.6 + paraformaldehyde 0.1%	3.35	3.55	3.51	3.58	3.65	3.85	3.58
K_2CO_3 1.5%	5.41	5.00	5.94	5.41	4.78	5.20	5.29
Foam sealants					3.62	4.10	3.68
K_2CO_3 1.5% + propionic acid 0.5%	4.96	4.46	5.41	4.96	4.28	4.82	4.82
H_2SO_4 to pH 3.6	3.82	3.61	4.10	3.52	3.50	4.15	3.78
Formic acid to pH 3.6	3.82	3.81	4.11	3.81	3.95	4.40	3.98
Dried molasses 3.0%	4.01	3.90	4.10	3.90	3.72	4.40	4.00

Table 5 (cont'd.)

Treatment	Day 2	8	15	22	32	76	$\bar{x}$
Sucrose-starch mix 3.0%	3.76	3.82	3.84	3.98	3.72	4.62	3.96
Sodium benzoate 0.1%	4.04	3.88	4.10	4.06	3.92	4.22	4.04
Lactobacillus casei + *L. bulgaricus*	3.85	3.84	4.02	4.24	4.05	4.68	4.11
Lactobacillus casei[b]	5.32	4.38	5.10	4.45	4.50	4.50	4.71
Wet brewers' grains without yeast[c]	4.10	4.10	4.17	4.23	4.00	4.55	4.19
Wet brewers' grains + 10% yeast[c]	4.17	4.02	4.53	4.08	3.79	4.32	4.15
Average	4.14	4.06	4.35	4.14	3.91	4.44	4.18

[a] averages of four values

[b] averages of two values

[c] averages of 70 values

Table 6. Analysis of variance of the pH of ensiled brewers' grains. Trial 1

Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Additives	15	4.2881	45.52**
Yeast	1	0.1526	1.62
(Additives) (Yeast)	15	0.5435	5.77**
Duplicates/trt. combination	32	0.0942	
Days	3	0.9768	11.63**
(Additives) (Days)	45	0.1356	1.61*
(Yeast) (Days)	3	0.8149	9.70**
(Additives) (Yeast) (Days)	45	0.1706	2.03**
Residual error	96	0.0840	

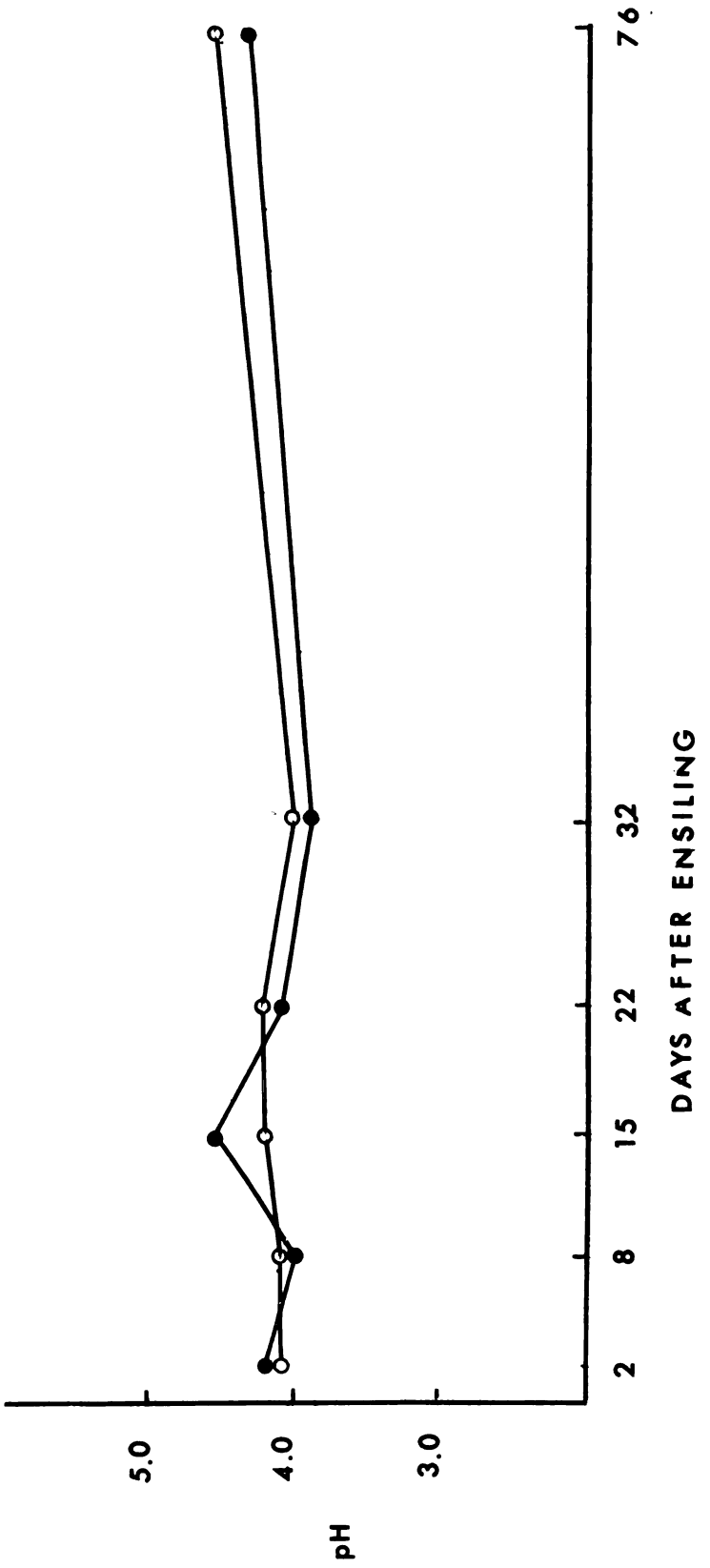
* Significant P<.05

** Significant P<.01

Figure 1. Change in pH of wet brewers' grains ensiled with or without yeast. Trial 1.

(o———o) wet brewers' grains without yeast

(●———●) wet brewers' grains plus 10% yeast


5.0
4.0
3.0
pH
2
8
15
22
32
76
DAYS AFTER ENSILING

higher than the pH of the grain treated with formic acid ($P<.01$). Formic acid plus paraformaldehyde lowered the pH more than formic acid alone ($P<.01$). Molasses and the sucrose-starch mix did not affect the pH when compared to the control and did not differ among themselves either. The pH of sodium benzoate and Lactobacillus culture treated grain was 4.04 and 4.11, respectively, which was not different from that of the control (4.03).

In all treatments, except the potassium carbonate treatment, pH was low enough to maintain silage in good condition provided anaerobic conditions were maintained.

Temperature. Temperatures of brewers' grains at different intervals during ensiling process are presented in Table 7. Buckets containing brewers' grains were stored at 70° to $84^{\circ}F$. Temperature of the grain inside buckets was above room temperature until day 8, and usually near room temperature thereafter.

Analysis of variance for temperature (Table 8) indicated that the effects of additives, yeast and days, and their interactions were highly significant. Temperatures of the grains with yeast added ($76.6^{\circ}F$) were lower than temperatures of the grains without yeast ($79.7^{\circ}F$). Treatment with yeast decreased temperature after day 8 (Figure 2).

Temperatures in buckets sealed with foam were significantly lower than in control (76.2 vs $79.8^{\circ}F$) ($P<.05$). Temperatures of the grains with basic treatments did not differ significantly from the control or from the acid

Table 7. Changes in temperature (F) with time in wet brewers' grains ensiled with different additives. Trial 1[a]

Treatment	Day													
	1	2	3	4	6	8	11	13	15	18	22	32	76[b]	$\bar{x}$
Control	75.0	76.2	73.2	72.8	79.8	82.5	81.2	80.5	83.0	85.5	82.5	78.0	86.5	79.8
Propionic acid (0.5%)	72.2	75.2	72.0	73.5	80.5	76.8	79.2	80.5	81.2	86.8	79.8	77.5	87.5	78.7
Propionic acid (1.0%)	67.8	76.2	72.8	74.0	79.8	79.8	81.2	83.8	85.5	91.2	84.2	78.2	84.5	79.9
Ammonium propionate (0.5%)	74.0	77.0	75.8	77.0	79.2	79.2	79.0	82.8	83.0	84.5	87.2	77.2	85.0	80.1
Ammonium isobutyrate (0.5%)	74.8	77.8	71.0	72.5	78.8	79.2	76.8	78.8	81.8	84.0	80.2	78.0	86.0	78.4
Ammonia (0.3% nitrogen)	75.0	78.2	73.5	73.8	78.0	76.8	76.8	80.5	81.8	84.0	81.0	79.2	86.0	78.8
Paraformaldehyde (0.1%)	75.5	77.0	72.5	74.0	78.2	78.5	74.8	79.0	81.2	84.8	79.2	77.2	87.0	78.4
Formic acid to pH 3.2 + paraformaldehyde (0.1%)	73.0	75.0	69.8	70.5	76.2	73.8	75.8	76.0	78.2	83.2	77.8	76.2	86.0	76.3
Potassium carbonate (1.5%)	77.0	76.5	72.5	72.5	79.0	77.2	81.0	81.0	83.5	86.0	79.8	76.5	85.0	79.0

Table 7 (cont'd.)

Treatment	Day 1	2	3	4	6	8	11	13	15	18	22	32	76[b]	$\bar{x}$
Foam sealants	76.0	74.5	72.0	69.8	77.2	73.5	77.2	74.2	78.0	81.0	77.5	76.2	84.0	76.2
K_2CO_3 + propionic acid (0.5%)	75.5	78.2	72.8	72.8	80.0	77.0	79.0	79.8	83.5	85.5	79.2	77.5	84.0	78.8
Sulfuric acid to pH 3.6	72.5	75.2	73.2	72.5	78.5	74.5	76.8	76.5	80.2	84.8	80.2	78.8	86.0	77.7
Formic acid to pH 3.6	72.5	74.5	71.8	70.2	75.5	75.2	78.8	78.5	82.8	82.2	79.2	76.2	87.5	77.5
Dried molasses	75.5	74.8	72.5	72.8	75.0	74.8	77.0	78.0	80.8	84.0	78.5	74.0	84.5	77.1
Sucrose-starch (3%)	77.0	75.5	73.5	72.5	74.5	74.5	76.0	77.5	79.5	83.5	78.0	77.8	86.0	77.4
Sodium benzoate (0.1%)	75.0	74.8	73.2	72.2	76.5	77.2	79.2	78.5	82.2	83.0	80.5	75.8	86.5	78.1
Lactobacillus casei + *L. bulgaricus*	72.5	76.0	73.0	71.8	74.5	73.5	78.8	76.2	80.2	84.0	79.2	76.8	88.5	77.3
Lactobacillus casei[b]	78.0	78.0	75.5	75.0	80.0	78.0	84.5	78.0	81.0	83.5	82.0	80.5	86.0	80.0
Grain without yeast[c]	75.3	77.6	73.7	73.6	79.4	79.2	80.8	80.9	83.8	86.9	80.6	78.0	87.1	79.8
Grain + 10% yeast[c]	73.0	74.5	71.6	71.6	76.1	74.1	75.7	77.0	79.4	83.3	79.1	76.3	84.4	76.6

Table 7 (cont'd.)

Treatment	Day													$\bar{x}$
	1	2	3	4	6	8	11	13	15	18	22	32	76[b]	
Average	74.4	76.2	72.8	72.8	77.8	76.8	78.5	78.9	81.5	84.7	80.4	77.3	85.9	78.3
Room temperature	72.0	70.0	75.0	74.0	73.0	76.0	80.0	82.0	84.0	80.0	74.0	74.0	77.0	76.2

[a] averages of four values

[b] averages of two values

[c] averages of 66 values

Table 8. Analysis of variance of the temperatures of ensiled brewers' grains. Trial 1

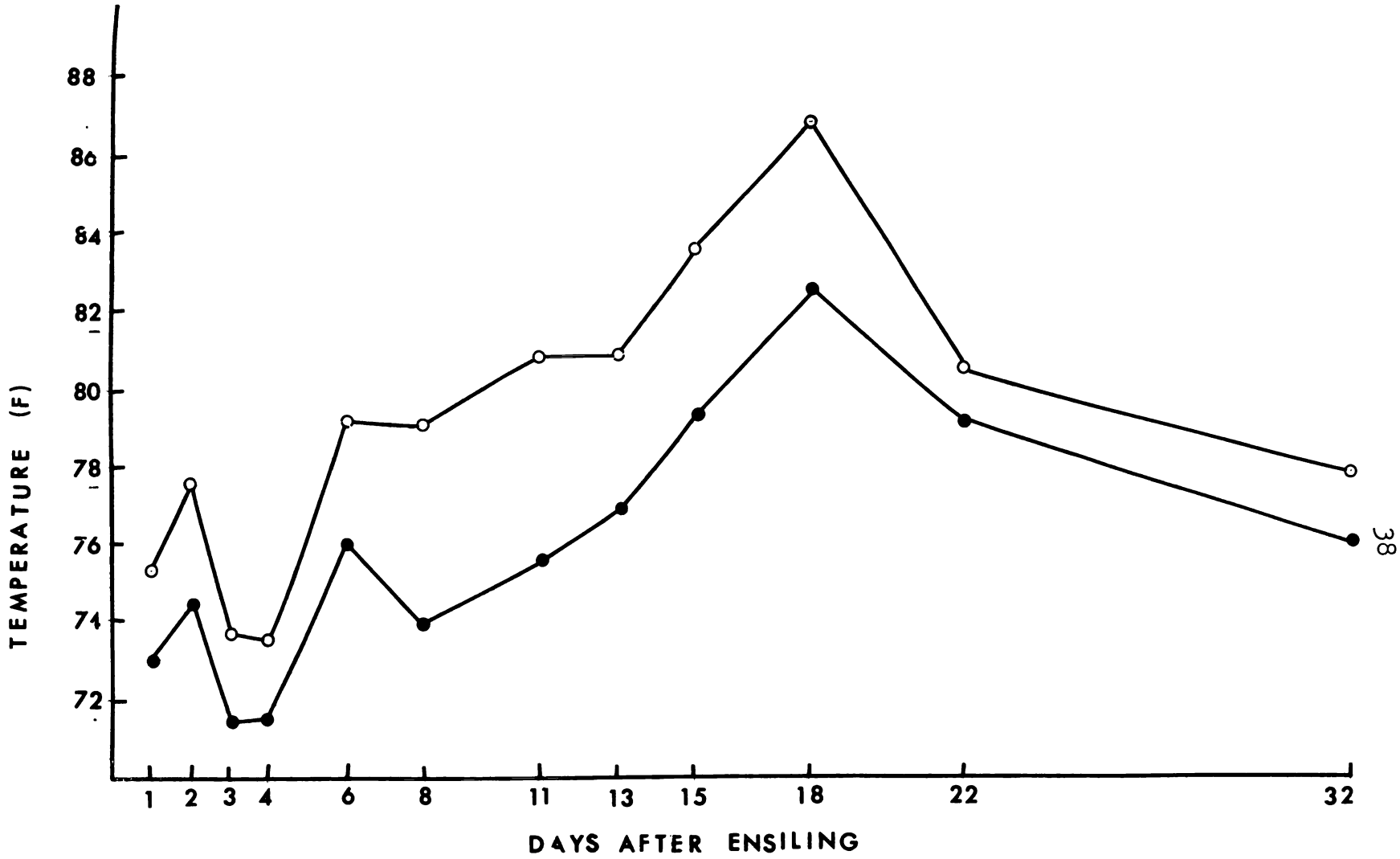
Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Additives	16	83.7108	7.14**
Yeast	1	2055.1777	175.29**
(Additives) (Yeast)	16	47.5162	4.05**
Duplicates/trt. combination	34	11.7243	
Days	11	892.0431	311.54**
(Additives) (Day)	176	9.4740	3.31**
(Yeast) (Day)	11	27.5814	9.63**
(Additives) (Yeast) (Day)	176	5.8774	2.05**
Residual error	374	2.8633	

** Significant P<.01

Figure 2. Change in temperature of wet brewers' grains ensiled with or without yeast. Trial 1.

(o———o) wet brewers' grains without yeast

(●———●) wet brewers' grains plus 10% yeast


TEMPERATURE (F)
88
86
84
82
80
78
76
74
72
1
2
3
4
6
8
11
13
15
18
22
32
DAYS AFTER ENSILING

treatments. Acid treated grains had higher average temperatures than those sealed with foam (78.1° vs 76.2°F) ($P<.10$). Temperatures of the grains treated with propionic acid, ammonium propionate or potassium carbonate plus propionic acid did not differ. Addition of formic acid lowered temperature more than did propionic acid (76.9° vs 79.3°F) ($P<.05$). Temperature of the grains treated with formic acid or with formic acid plus paraformaldehyde were similar, 77.5° and 76.3°F. Temperatures of grains treated with molasses (77.1°F) or sucrose-starch mix (77.4°F) did not differ significantly from those sealed with foam (76.2°F), nor among themselves. Grains treated with sodium benzoate had lower temperatures than did the control (78.1° vs 79.7°F) ($P<.10$). There was no significant difference in average temperature between grains sealed with foam and those treated with Lactobacillus culture (76.2° vs 77.3°F).

Dry matter and protein. The average dry matter content on day 32 was 22.4% and decreased to 20.5% after 76 days (Table 9). Only sodium benzoate treated grains did not have this decrease. During this interval the protein content of the dry matter increased in every treatment except in the one with formic acid plus paraformaldehyde. The average was 34.3% on day 32 and 40.9% on day 76.

The changes in dry matter and protein were similar for the grains alone or grains plus yeast.

Acid detergent fiber and acid detergent insoluble nitrogen. Average acid detergent fiber increased from 22.8%

Table 9. Dry matter and protein content of ensiled wet brewers' grains. Trial 1[a]

Treatment	% dry matter			% protein (dry matter basis)		
	after 32 days	after 76 days	$\bar{x}$	after 32 days	after 76 days	$\bar{x}$
Control	22.6	20.7	21.7	33.3	38.7	36.0
Propionic acid (0.5%)	21.7	20.6	21.2	32.3	38.3	35.6
Propionic acid (1.0%)	23.6	21.1	22.4	33.9	38.0	36.0
Ammonium propionate (0.5%)	23.4	20.6	22.0	36.3	42.6	38.8
Ammonium isobutyrate (0.5%)	22.7	19.4	21.0	33.9	45.1	39.5
Ammonia (0.3% nitrogen)	21.7	19.1	20.4	35.8	41.2	38.5
Paraformaldehyde (0.1%)	19.4	19.6	19.5	37.1	41.7	39.4
Formic acid to pH 3.2 + paraformaldehyde (0.1%)	23.7	22.6	23.2	35.1	35.5	35.3
K_2CO_3 (1.5%)	22.2	20.4	21.3	33.2	43.7	38.4
Foam sealants	23.6	19.8	21.7	32.7	41.8	37.2
K_2CO_3 (1.5%) + Propionic acid (0.5%)	22.7	20.0	21.4	29.8	42.5	36.2

Table 9 (cont'd.)

Treatment	% dry matter		$\bar{x}$	% protein (dry matter basis)		$\bar{x}$
	after 32 days	after 76 days		after 32 days	after 76 days	
H_2SO_4 to pH 3.6	21.4	18.3	19.8	33.7	44.8	39.2
Formic acid to pH 3.6	23.4	22.4	22.9	33.7	38.1	35.9
Dried molasses (3.0%)	24.5	22.0	23.2	32.0	38.0	35.0
Sucrose-starch (3.0%)	22.8	21.3	22.0	33.6	40.4	37.0
Sodium benzoate (0.1%)	21.9	22.4	22.2	36.5	38.5	37.5
Lactobacillus casei + L. bulgaricum	20.4	18.8	19.6	37.3	41.8	40.0
Lactobacillus casei[b]	21.1	19.2	20.2	35.9	45.2	40.6
Wet brewers' grains without yeast[c]	21.8	20.5	21.1	34.2	41.3	37.8
Wet brewers' grains + 10% yeast[c]	23.0	20.5	21.7	34.2	40.3	37.2
Average	22.4	20.5	21.4	34.3	40.9	37.6

[a] average of two values

[b] one value

[c] average of 35 values

on day 32 to 25.3% on day 76. The highest value was for the treatment with dried molasses (Table 10). Addition of yeast decreased the acid detergent fiber content in the grains.

Acid detergent insoluble nitrogen increased from 0.92% on day 32 to 0.97% on day 76 after ensiling. Addition of yeast decreased the acid detergent insoluble nitrogen of the grains by an average of 9%. The highest values were for the treatments that had paraformaldehyde. These treatments had from 17 to 30% higher acid detergent insoluble nitrogen content than controls. This is due to the fact that formaldehyde binds proteins making them insoluble. In the case of ruminants this complex is not digested in the rumen but may be digested in the abomassum.

Ethanol. Ethanol content of the silage attained maximum concentration after 15 days of ensiling. From day 15 to day 32 ethanol concentration decreased, but increased on day 76 to a level of 48% of that on day 15. Ethanol content of the silage with yeast (0.62%) was greater than without yeast (0.48%) ($P<.05$) (Table 11). The interaction of additives with days and of days with yeast was significant (Table 12). The control did not differ from acid or basic treatments in ethanol content. The ethanol content of silages treated with acids or bases was similar. Treatments with propionic acid or ammonium propionate had an average ethanol concentration not different from the control (0.29% vs 0.34%). Formic acid alone was similar to formic acid plus paraformaldehyde in its effect on ethanol formation when compared

Table 10. Acid Detergent Fiber (ADF) and Acid Detergent insoluble Nitrogen (ADN) content of ensiled wet brewers' grains. Trial 1[a]

Treatment	% ADF (% DM)			% ADN (% DM)		
	after 32 days	after 76 days	x	after 32 days	after 76 days	$\bar{x}$
Control	21.9	24.7	23.3	0.94	0.88	0.91
Propionic acid (0.5%)	21.2	25.5	23.4	0.86	1.02	0.94
Propionic acid (1.0%)	23.2	24.4	23.8	0.78	0.92	0.84
Ammonium propionate (0.5%)	24.4	25.7	25.1	0.90	0.87	0.88
Ammonium isobutyrate (0.5%)	21.7	26.6	24.2	0.85	0.95	0.90
Ammonia (0.3% nitrogen)	22.5	25.2	23.8	0.88	0.94	0.91
Paraformaldehyde (0.1%)	23.7	27.0	25.3	1.04	1.23	1.13
Formic acid to pH 3.2 + paraformaldehyde (0.1%)	22.4	24.8	23.6	1.18	1.34	1.26
K_2CO_3 (1.5%)	23.1	27.6	25.3	1.00	1.00	1.00
Foam sealants	23.7	25.3	24.5	0.92	1.07	0.99
K_2CO_3 (1.5%) + propionic acid (0.5%)	20.8	24.7	22.7	0.90	0.86	0.88

Table 10 (cont'd.)

Treatment	% ADF (% DM) after 32 days	% ADF (% DM) after 76 days	$\bar{x}$	% ADN (% DM) after 32 days	% ADN (% DM) after 76 days	$\bar{x}$
H_2SO_4 to pH 3.6	21.5	24.1	22.8	0.91	1.06	0.99
Formic acid to pH 3.6	21.5	22.5	22.0	0.82	0.90	0.86
Dried molasses (3.0%)	25.8	28.5	27.1	1.02	0.87	0.84
Sucrose-starch (3.0%)	22.9	24.8	23.9	0.78	0.80	0.79
Sodium benzoate (0.1%)	23.6	25.2	24.4	0.94	0.86	0.90
Lactobacillus casei + L. bulgaricus	23.3	24.0	23.7	0.91	0.87	0.89
Lactobacillus casei[b]	24.7	26.4	25.6	1.05	0.98	1.01
Wet brewers' grains + 10% yeast[c]	22.2	24.3	23.2	0.90	0.88	0.89
Wet brewers' grains without yeast[c]	23.5	26.3	24.9	0.94	1.00	0.97
Average	22.8	25.3	24.1	0.92	0.97	0.94

[a] average of two values

[b] one value

[c] average of 35 values

Table 11. Changes in ethanol concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 1[a]

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Control	0.07	0.60	0.22	0.57	0.08	0.49	0.34
Propionic acid (0.5%)	0.15	1.65	0.33	1.08	0.28	0.34	0.34
Propionic acid (1.0%)	0.00	0.08	0.05	0.21	0.11	0.47	0.15
Ammonium propionate (0.5%)	0.04	0.19	0.04	0.22	0.14	0.45	0.18
Ammonium isobutyrate (0.5%)	0.42	0.82	0.46	0.69	0.13	0.56	0.55
Ammonia (0.3% nitrogen)	0.64	0.48	0.33	0.92	0.16	0.33	0.48
Paraformaldehyde (0.1%)	0.83	0.57	0.48	0.70	0.16	0.17	0.49
Formic acid to pH 3.6 + paraformaldehyde (0.1%)	0.12	0.73	0.66	0.39	0.11	0.23	0.37
K_2CO_3 (1.5%)	0.44	0.78	0.62	0.24	0.14	0.34	0.43
Foam sealants					0.20	1.10	0.65
K_2CO_3 (1.5%) + propionic acid (0.5%)	0.77	0.56	0.52	0.20	0.04	0.54	0.49
H_2SO_4 to pH 3.6	1.60	0.63	1.44	0.63	0.07	0.28	0.78
Formic acid to pH 3.6	0.96	0.17	1.30	0.38	0.06	0.50	0.56
Dried molasses (3.0%)	1.54	0.32	1.92	0.32	0.07	0.65	0.80

Table 11 (cont'd.)

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Sucrose-starch mix (3.0%)	2.12	1.38	2.50	0.94	0.07	0.64	1.26
Sodium benzoate (0.1%)	0.89	0.38	1.32	0.47	0.05	0.48	0.60
Lactobacillus casei + *L. bulgaricus*	1.71	0.38	2.00	0.37	0.03	0.60	0.85
Lactobacillus casei[b]	0.14	0.07	0.64	0.26	0.36	0.46	0.32
Wet brewers' grains without yeast[c]	0.58	0.47	0.88	0.49	0.13	0.33	0.48
Wet brewers' grains + 10% yeast[c]	0.96	0.74	0.88	0.55	0.09	0.51	0.62
Average	0.77	0.61	0.88	0.52	0.11	0.42	0.55

[a] averages of four values

[b] averages of two values

[c] averages of 66 values

Table 12. Analysis of variance of ethanol content of ensiled wet brewers' grains. Trial 1

Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Additives	15	2.6554	7.05**
Yeast	1	1.8735	4.98*
(Additives) (Yeast)	15	0.1851	0.49
Duplicates/trt. combination	32	0.3765	
Days	3	1.6652	13.28**
(Additives) (Days)	45	0.8335	6.65**
(Yeast) (Days)	3	0.5013	4.00*
(Additives) (Yeast) (Days)	45	0.1547	1.23
Residual error	96	0.1254	

* Significant P<.05

** Significant P<.01

until day 22 of ensiling. During the same period of time grains treated with sulfuric acid had a higher ethanol content than did control ($P<.05$). Addition of molasses or sucrose-starch mix increased the ethanol content over the control ($P<.01$). The treatment with the sucrose-starch mix had a higher ethanol content than the treatment with molasses ($P<.05$). The addition of sodium benzoate did not increase the ethanol concentration when compared with the control, but lactic acid culture inoculation resulted in an increased ethanol formation ($P<.05$).

After 32 days of ensiling ethanol content of the grains had decreased to levels close to 0.10% for all treatments, except 0.5% propionic acid and Lactobacillus casei culture which had concentrations of 0.28 and 0.36%, respectively.

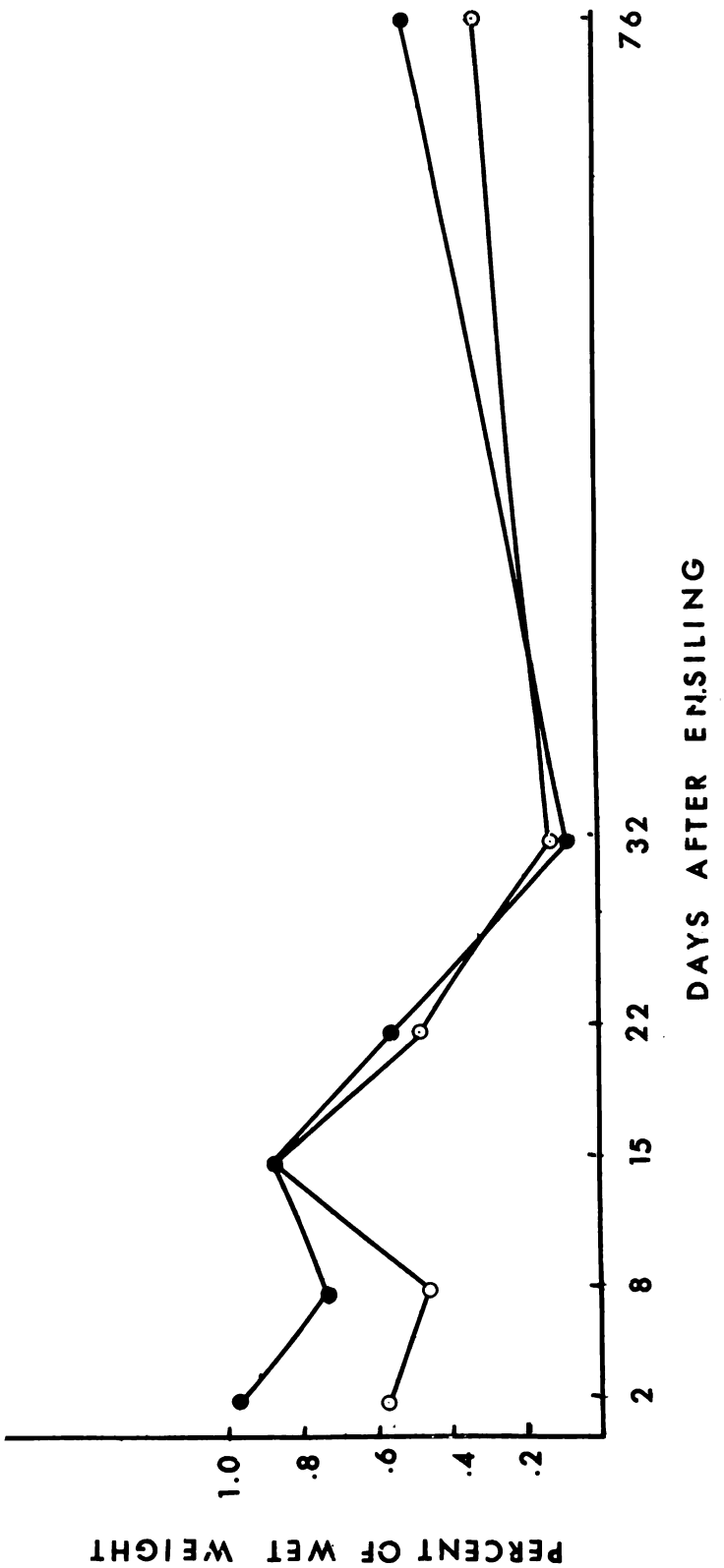
At the end of 76 days the grains that had most ethanol were those sealed with foam (1.10%). Grains to which molasses, a sucrose-starch mix or a Lactobacillus casei plus L. bulgaricus culture was added averaged more ethanol than did control (0.63% vs 0.49%). Low levels were observed at this time in the grains treated with paraformaldehyde (0.17%), formic acid plus paraformaldehyde (0.23%) or sulfuric acid (0.28%) when compared to the average of all treatments (0.42%). Grains with yeast had a higher ethanol concentration than grains without yeast (0.51% vs 0.33%) (Figure 3).

Acetic acid. Analysis of variance for acetic acid values on days 2, 8, 15 and 22 indicated that the effects of additives, yeast and days, and their interactions were significant

Figure 3. Change in ethanol content of wet brewers' grains ensiled with or without yeast. Trial 1.

(o——o) wet brewers' grains without yeast

(●——●) wet brewers' grains plus 10% yeast


PERCENT OF WET WEIGHT
1.0
.8
.6
.4
.2
2
8
15
22
32
76
DAYS AFTER ENSILING

(Table 14). Comparisons of the treatment means on these days indicated the following: Grains treated with acids (propionic, formic and sulfuric) had lower acetic acid content when compared to the control ($P<.01$). Basic treatments (ammonia and potassium carbonate) resulted in lower concentrations than the control ($P<.01$), but higher than acid treatments ($P<.05$). Grains treated with propionic acid alone did not differ from those treated with ammonium propionate, potassium carbonate plus propionic acid or formic acid. Formic acid alone had a similar effect than formic acid plus paraformaldehyde. However, the grains treated with formic acid were lower in acetic acid content than the control ($P<.01$). Addition of molasses, a sucrose-starch mixture or sodium benzoate did not change the acetic acid content of the silage when compared to the control. The grains to which a Lactobacillus casei plus L. bulgaricus culture was added had a lower concentration than the control ($P<.05$).

During the first 15 days acetic acid concentrations were about the same for grains with and without yeast, but from day 22 the grains with yeast had higher concentrations (Figure 4). On day 32 grains without yeast had 0.52% acetic acid and grains with yeast 0.30%. The concentration increased after 76 days of ensiling, but again grain without yeast had more acetic acid than grain with yeast (0.74% vs 0.41%).

After 32 days of ensiling grains (Table 13) treated with ammonium propionate, potassium carbonate, dried molasses and a sucrose-starch mix had higher acetic acid concentrations

Table 13. Changes in acetic acid concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 1[a]

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Control	0.43	0.56	0.26	1.06	0.56	0.72	0.60
Propionic acid (0.5%)	0.16	0.20	0.18	0.31	0.35	0.52	0.29
Propionic acid (1.0%)	0.21	0.23	0.26	0.62	0.41	0.58	0.38
Ammonium propionate (0.5%)	0.16	0.21	0.40	0.58	0.90	0.63	0.48
Ammonium isobutyrate (0.5%)	0.18	0.18	0.26	0.26	0.18	0.50	0.26
Ammonia (0.3% nitrogen)	0.16	0.23	0.35	0.28	0.48	0.45	0.32
Paraformaldehyde (0.1%)	0.19	0.13	0.23	0.22	0.18	0.44	0.23
Formic acid to pH 3.6 + paraformaldehyde (0.1%)	0.04	0.21	0.15	0.10	0.16	0.25	0.15
K_2CO_3 (1.5%)	0.23	0.30	0.42	0.68	0.74	1.06	0.57
Foam sealants					0.22	0.50	0.36
K_2CO_3 (1.5%) + propionic acid (0.5%)	0.32	0.42	0.43	0.24	0.16	0.59	0.36
H_2SO_4 to pH 3.6	0.24	0.34	0.17	0.24	0.14	0.55	0.28
Formic acid to pH 3.6	0.11	0.22	0.15	0.14	0.10	0.59	0.22
Dried molasses (3.0%)	0.28	0.88	0.37	0.28	0.66	0.42	0.48

Table 13 (cont'd.)

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Sucrose-starch mix (3.0%)	0.30	0.56	0.38	0.35	0.98	0.63	0.53
Sodium benzoate (0.1%)	0.36	0.52	0.42	0.53	0.36	0.88	0.51
Lactobacillus casei + *L. bulgaricus*	0.16	0.24	0.16	0.27	0.46	0.52	0.30
Lactobacillus casei[b]	0.04	0.34	0.45	0.40	0.30	0.66	0.36
Wet brewers' grains without yeast[c]	0.21	0.31	0.29	0.52	0.52	0.74	0.43
Wet brewers grains + 10% yeast[c]	0.23	0.37	0.29	0.25	0.30	0.41	0.31
Average	0.22	0.34	0.29	0.39	0.41	0.58	0.37

[a] averages of four values

[b] averages of two values

[c] averages of 66 values

Table 14. Analysis of variance of acetic acid content of wet brewers' grains. Trial 1

Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Additives	15	0.2616	5.40**
Yeast	1	0.1506	3.11*
(Additives) (Yeast)	15	0.1072	2.21*
Duplicates/trt. combination	32	0.0485	
Days	3	0.3251	14.67**
(Additives) (Days)	45	0.0774	3.49**
(Yeast) (Days)	3	0.3607	16.28**
(Additives) (Yeast) (Days)	45	0.0571	2.57**
Residual error	96	0.0222	

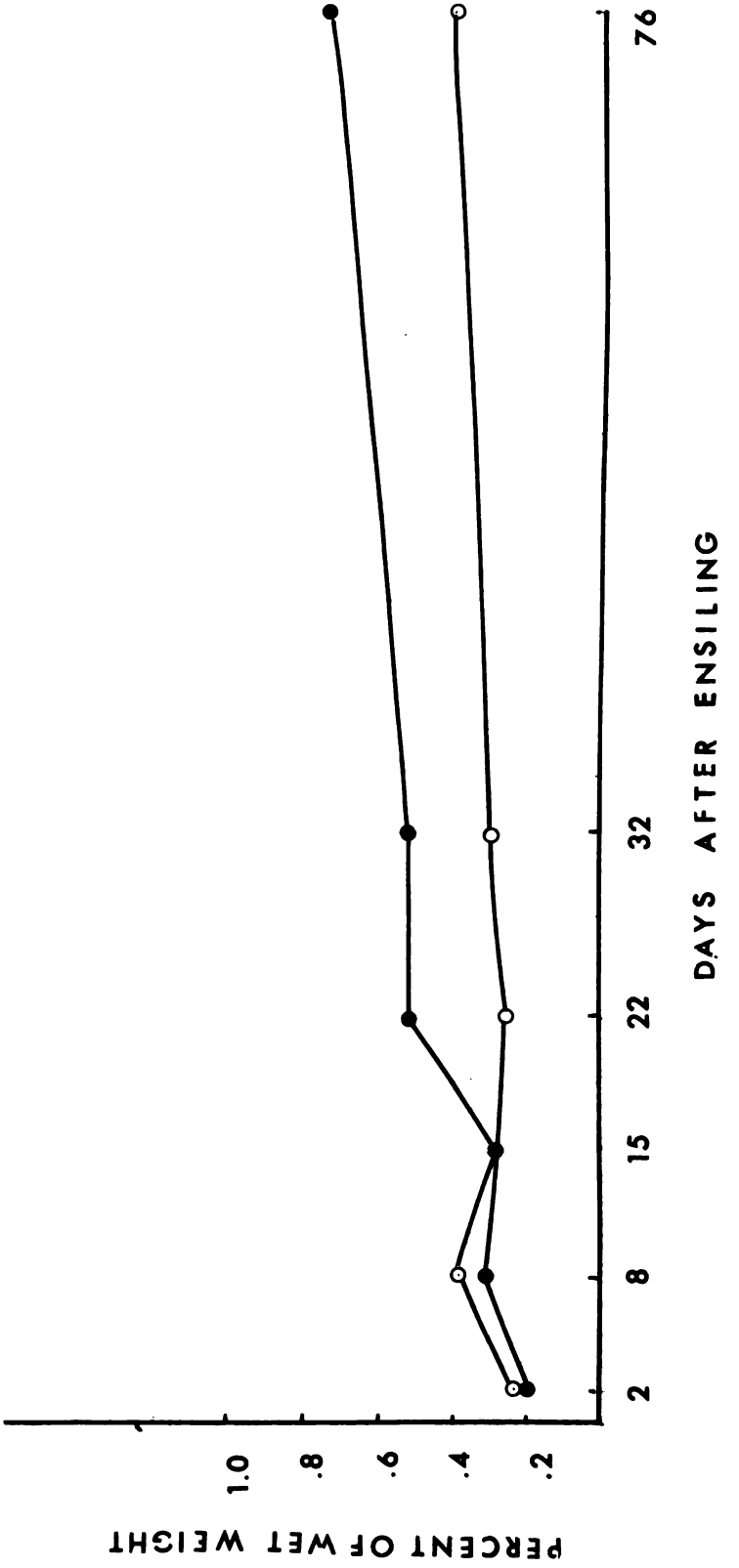
* Significant P<.10

** Significant P<.01

Figure 4. Change in acetic acid content of wet brewers' grains ensiled with or without yeast. Trial 1.

(o———o) wet brewers' grains without yeast

(●———●) Wet brewers' grains plus 10% yeast


PERCENT OF WET WEIGHT
1.0
.8
.6
.4
.2
2
8
15
22
32
76
DAYS AFTER ENSILING

than the control (0.82% vs 0.56%). Low levels (0.14 to 0.22%) were found in the grains treated with ammonium isobutyrate, paraformaldehyde, formic acid plus paraformaldehyde, potassium carbonate plus propionic acid, sulfuric acid, formic acid and in the grain sealed with foam.

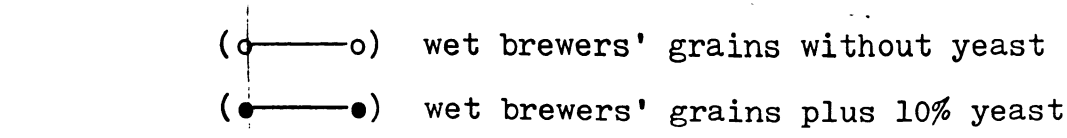
After 76 days only the grains treated with potassium carbonate and sodium benzoate had acetic acid concentrations higher than the control (1.06 and 0.88% vs 0.72%). Treatment with formic acid plus paraformaldehyde resulted in a low acetic acid concentration (0.25%) which compares with 0.72% for the control and 0.50% for the sealed grains.

In general, addition of acid or basic chemical compounds resulted in a decrease in acetic acid content in the silage, but the addition of sugars did not have any effect after 32 days. However, on day 76 only formic acid plus paraformaldehyde lowered acetic acid concentration of the grain (Table 13).

Propionic acid. Propionic acid was low in all grains during the first 15 days of the ensiling process, but started to increase at day 22 until day 76 when the average was 0.40% (Figure 5). Ensiled wet brewers' grains without yeast had greater propionic acid content ($P<.01$) than did grains to which 10% yeast was added (Table 16).

On day 32 silage from grain treated with ammonia, formic acid, paraformaldehyde, formic acid plus paraformaldehyde, sulfuric acid, dried molasses, sucrose-starch mix, sodium benzoate, lactic acid culture or sealed with foam, had low concentrations of propionic acid (0.02 to 0.08%). Higher (Table 15)

Figure 5. Change in propionic acid content of wet brewers' grains ensiled with or without yeast. Trial 1.



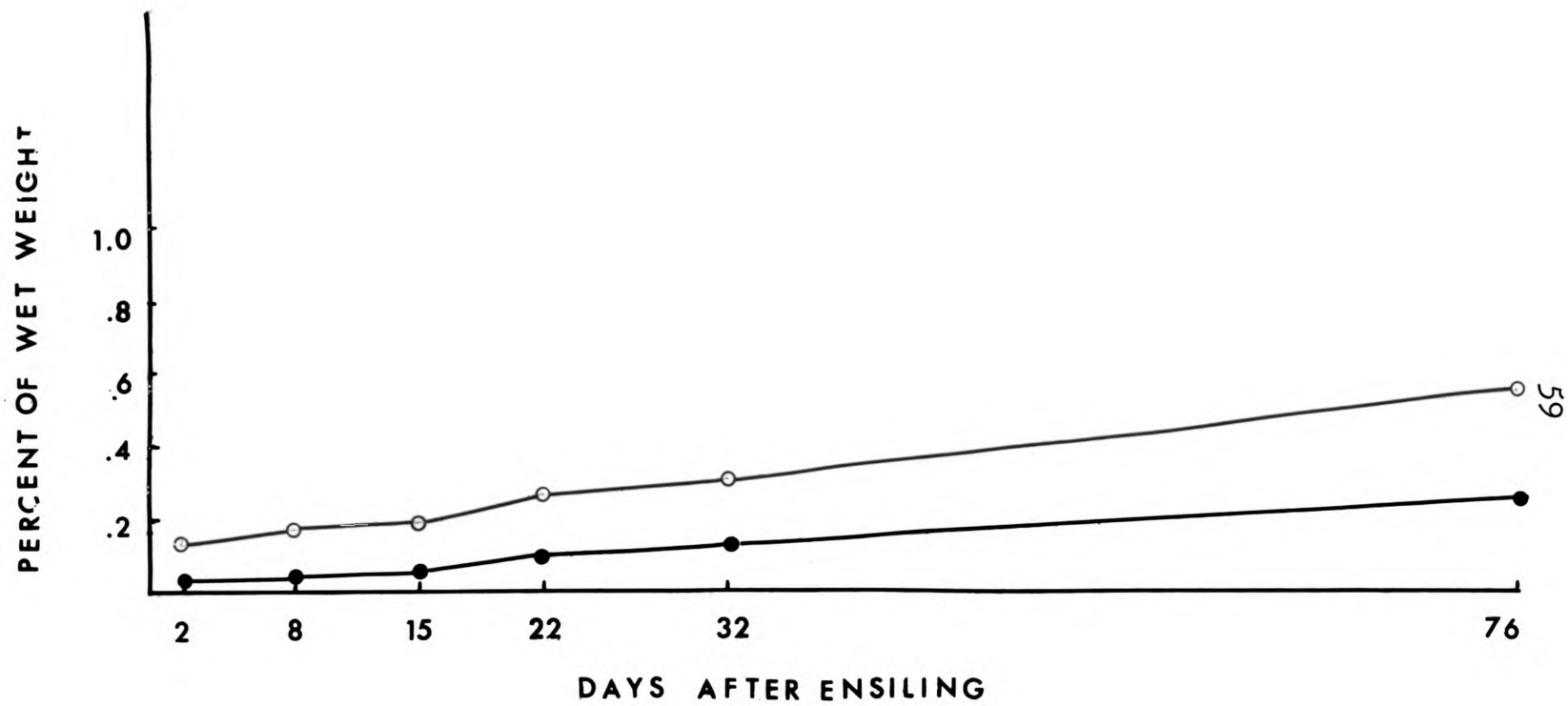

PERCENT OF WET WEIGHT
1.0
.8
.6
.4
.2
2
8
15
22
32
76
DAYS AFTER ENSILING

Table 15. Changes in propionic acid concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 1[a]

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Control	0.00	0.00	0.01	0.21	0.26	0.33	0.14
Propionic acid (0.5%)	0.12	0.21	0.22	0.28	0.30	0.40	0.26
Propionic acid (1.0%)	0.71	0.87	0.67	0.74	1.02	1.19	0.87
Ammonium propionate (0.5%)	0.04	0.06	0.08	0.17	0.36	0.46	0.24
Ammonium isobutyrate (0.5%)	0.12	0.16	0.16	0.36	0.30	0.76	0.31
Ammonia (0.3% nitrogen)	0.01	0.00	0.02	0.07	0.07	0.40	0.10
Paraformaldehyde (0.1%)	0.00	0.00	0.02	0.03	0.02	0.32	0.06
Formic acid to pH 3.6 + paraformaldehyde (0.1%)	0.01	0.01	0.00	0.01	0.04	0.08	0.03
K_2CO_3 (1.5%)	0.01	0.01	0.21	0.43	0.36	0.79	0.30
Foam sealants					0.04	0.34	0.19
K_2CO_3 (1.5%) + propionic acid (0.5%)	0.36	0.37	0.43	0.29	0.67	0.58	0.45
H_2SO_4 to pH 3.6	0.01	0.00	0.01	0.01	0.02	0.18	0.04
Formic acid to pH 3.6	0.04	0.04	0.08	0.05	0.06	0.18	0.07
Dried molasses (3.0%)	0.00	0.01	0.00	0.04	0.02	0.27	0.06

Table 15 (cont'd.)

Treatment	Day						$\bar{x}$
	2	8	15	22	32[b]	76[b]	
Sucrose-starch mix (3.0%)	0.01	0.00	0.00	0.07	0.04	0.26	0.06
Sodium benzoate (0.1%)	0.01	0.01	0.00	0.08	0.06	0.22	0.06
Lactobacillus casei + *L. bulgaricus*	0.00	0.00	0.00	0.15	0.08	0.45	0.11
Lactobacillus casei[b]	0.00	0.04	0.01	0.02	0.29	0.10	0.08
Wet brewers' grains without yeast[c]	0.14	0.18	0.19	0.27	0.31	0.56	0.28
Wet brewers' grains + 10% yeast[c]	0.04	0.04	0.05	0.10	0.13	0.26	0.10
Average	0.09	0.11	0.12	0.19	0.22	0.41	0.19

[a] averages of four values

[b] averages of two values

[c] averages of 66 values

Table 16. Analysis of variance of propionic acid content of wet brewers' grains. Trial 1

Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Additives	15	0.5984	219.76**
Yeast	1	1.1732	430.83**
(Additives) (Yeast)	15	0.4605	169.11**
Duplicates/trt. combination	32	0.0027	
Days	3	0.1095	18.24**
(Additives) (Days)	45	0.0170	2.84**
(Yeast) (Days)	3	0.0106	1.76
(Additives) (Yeast) (Days)	45	0.0276	4.59**
Residual error	96	0.0060	

** Significant P<.01

levels were found in the grains treated with propionic acid and in the control that had 0.26%.

On day 76 grain treated with formic acid plus paraformaldehyde had the lowest propionic acid content (0.08%), while high values were determined on samples from grains treated with propionic acid. Grain to which ammonium isobutyrate, ammonia, potassium carbonate or a lactic acid culture (L. casei plus L. bulgaricus) was added had propionic acid concentrations from 0.40 to 0.79% while the control had 0.33%.

Butyric acid. During the first 15 days of the experiment butyric acid concentrations in the grains were low (0.11% at day 15), but then started to increase and reached a maximum on day 76 (0.98%) (Table 17). Grains with yeast had less butyric acid than grain without yeast until day 32, nevertheless, concentrations at the end of the experiment were similar (Figure 6). Butyric acid was not detected in grains ensiled with 1% propionic acid until day 32. However, on day 76 this silage had a concentration of 0.42%. This value was the lowest for all treatments. Low values on day 32 were also observed in the grains treated with 0.5% propionic acid, paraformaldehyde, potassium carbonate plus propionic acid, formic acid, sulfuric acid, dried molasses and sucrose-starch mix. On day 76 only the grains treated with 1% propionic acid had butyric acid contents lower than control (0.42 vs 0.66%).

Grains ensiled by sealing with foam had 0.82% butyric acid after 76 days. Despite their butyric acid content,

Table 17. Changes in butyric acid concentration (percent in a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 1[a]

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Control	0.00	0.03	0.04	0.28	0.24	0.66	0.21
Propionic acid (0.5%)	0.00	0.00	0.01	0.11	0.15	0.72	0.16
Propionic acid (1.0%)	0.00	0.02	0.00	0.00	0.00	0.42	0.07
Ammonium propionate (0.5%)	0.00	0.02	0.06	0.26	0.34	0.88	0.26
Ammonium isobutyrate (0.5%)	0.00	0.02	0.13	0.34	0.40	1.28	0.24
Ammonia (0.3% nitrogen)	0.00	0.00	0.06	0.34	0.30	1.07	0.24
Paraformaldehyde (0.1%)	0.00	0.00	0.12	0.38	0.11	1.32	0.26
Formic acid to pH 3.6 + paraformaldehyde (0.1%)	0.00	0.15	0.00	0.00	0.42	0.70	0.21
K_2CO_3 (1.5%)	0.00	0.12	0.90	1.20	1.00	1.64	0.81
Foam sealants					0.10	0.82	0.46
K_2CO_3 (1.5%) + propionic acid (0.5%)	0.00	0.40	0.34	0.48	0.18	1.36	0.46
H_2SO_4 to pH 3.6	0.00	0.02	0.03	0.32	0.10	0.62	0.18
Formic acid to pH 3.6	0.00	0.07	0.00	0.32	0.20	1.04	0.27
Dried molasses (3.0%)	0.00	0.07	0.00	0.93	0.14	1.16	0.38

Table 17 (cont'd.)

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Sucrose-starch mix (3.0%)	0.00	0.04	0.00	1.05	0.21	1.21	0.42
Sodium benzoate (0.1%)	0.00	0.04	0.00	0.62	0.28	0.86	0.30
Lactobacillus casei + L. bulgaricus	0.00	0.06	0.00	0.74	0.58	1.02	0.40
Lactobacillus casei[b]	0.00	0.18	0.02	0.46	1.16	0.85	0.44
Wet brewers' grains without yeast[c]	0.00	0.09	0.12	0.57	0.38	0.99	0.36
Wet brewers' grains + 10% yeast[c]	0.00	0.04	0.09	0.35	0.22	0.98	0.28
Average	0.00	0.07	0.11	0.46	0.31	0.98	0.32

[a] averages of four values

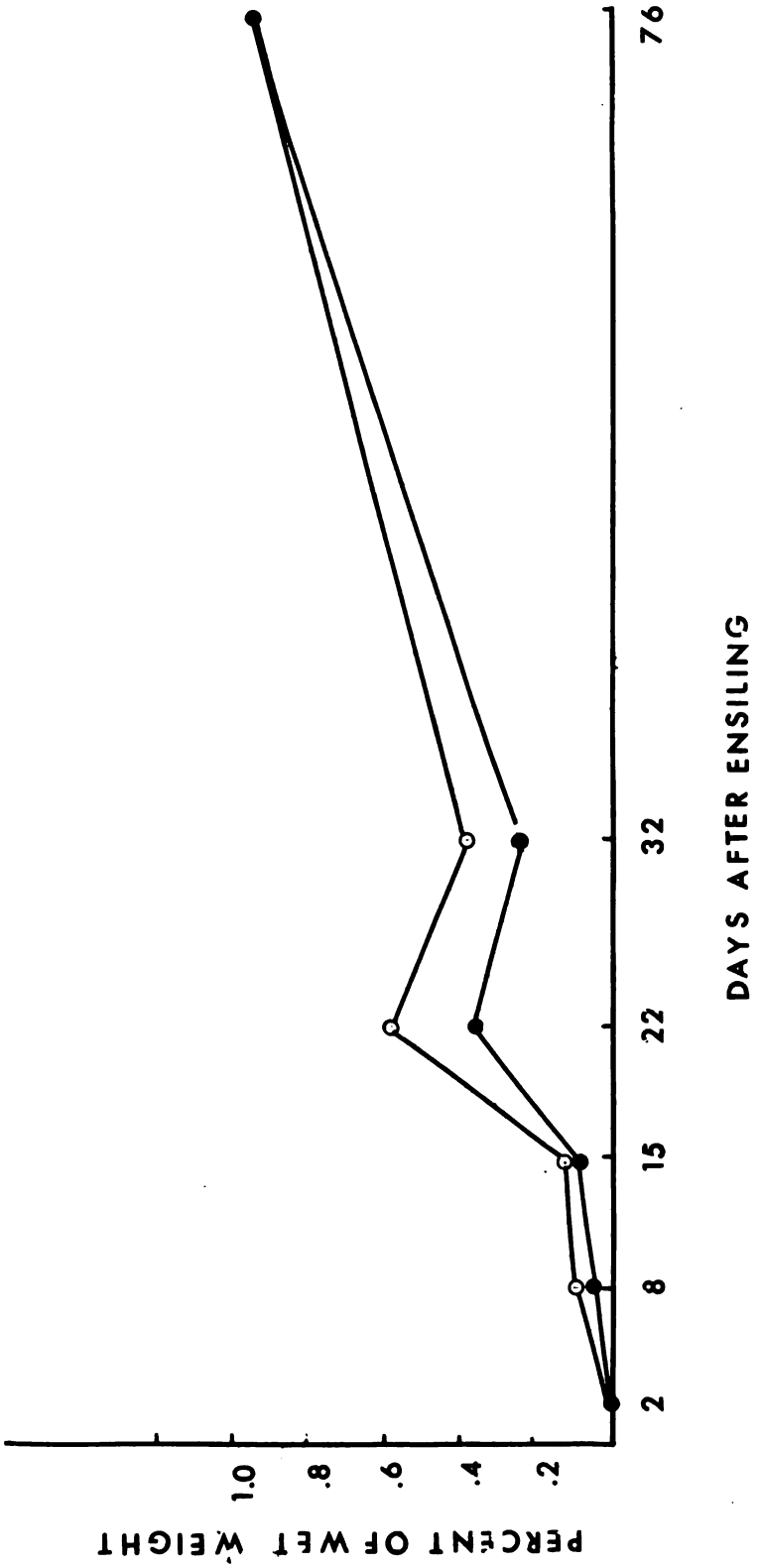
[b] averages of two values

[c] averages of 66 values

Figure 6. Change in butyric acid content of wet brewers' grains ensiled with or without yeast. Trial 1.

(o——o) wet brewers' grains without yeast

(●——●) wet brewers' grains plus 10% yeast


PERCENT OF WET WEIGHT
1.0
.8
.6
.4
.2
2
8
15
22
32
76
DAYS AFTER ENSILING

these grains had a good general appearance and their smell was not offensive and best of all treatments.

From day 2 to day 32 the effects of additives, yeast and days were significant ($P<.01$) on butyric acid concentration of brewers' grains (Table 18).

Lactic acid. Average lactic acid concentrations reached the highest level after eight days of ensiling (0.70%) and then decreased slowly until day 76. During the first 15 days, lactic acid was lower in the grain with 10% yeast, but after day 22 it was higher than in the grain without yeast (Figure 7). Until day 22 the difference in lactic acid content between the grains with and without yeast was not significant (Table 19). On day 32 concentrations were also similar (0.67 vs 0.65%), but on day 76 grains with yeast had about twice as much lactic acid as the grains without yeast (0.56 vs 0.25%).

Statistical analysis of the changes in lactic acid content of the grains on days 2, 8, 15 and 22 gave the following results: (1) The control did not differ from the grains treated with acid compounds (propionic, formic or sulfuric acid). (2) Addition of bases (ammonia or potassium carbonate) resulted in an increase when compared to the control or to the acid treated grains ($P<.01$). (3) Treatment with propionic acid resulted in lower lactic acid concentrations in the grains than treatment with ammonium propionate or potassium carbonate plus propionic acid ($P<.05$). (4) Grains treated with propionic acid did not differ in lactic acid content from those treated with formic acid. (5) Lactic acid concentrations

Table 18. Analysis of variance of butyric acid content of wet brewers' grains. Trial 1

Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Additives	15	0.2981	15.87**
Yeast	1	0.3496	18.60**
(Additives) (Yeast)	15	0.0454	2.41*
Duplicates/trt. combination	32	0.0188	
Days	3	2.7092	147.85**
(Additives) (Days)	45	0.1540	8.40**
(Yeast) (Days)	3	0.1519	8.29**
(Additives) (Yeast) (Days)	45	0.0362	1.97**
Residual error	96	0.0183	

* Significant P<.05

** Significant P<.01

Figure 7. Change in lactic acid content of wet brewers' grains ensiled with or without yeast. Trial 1.

(o———o) wet brewers' grains without yeast

(●———●) wet brewers' grains plus 10% yeast

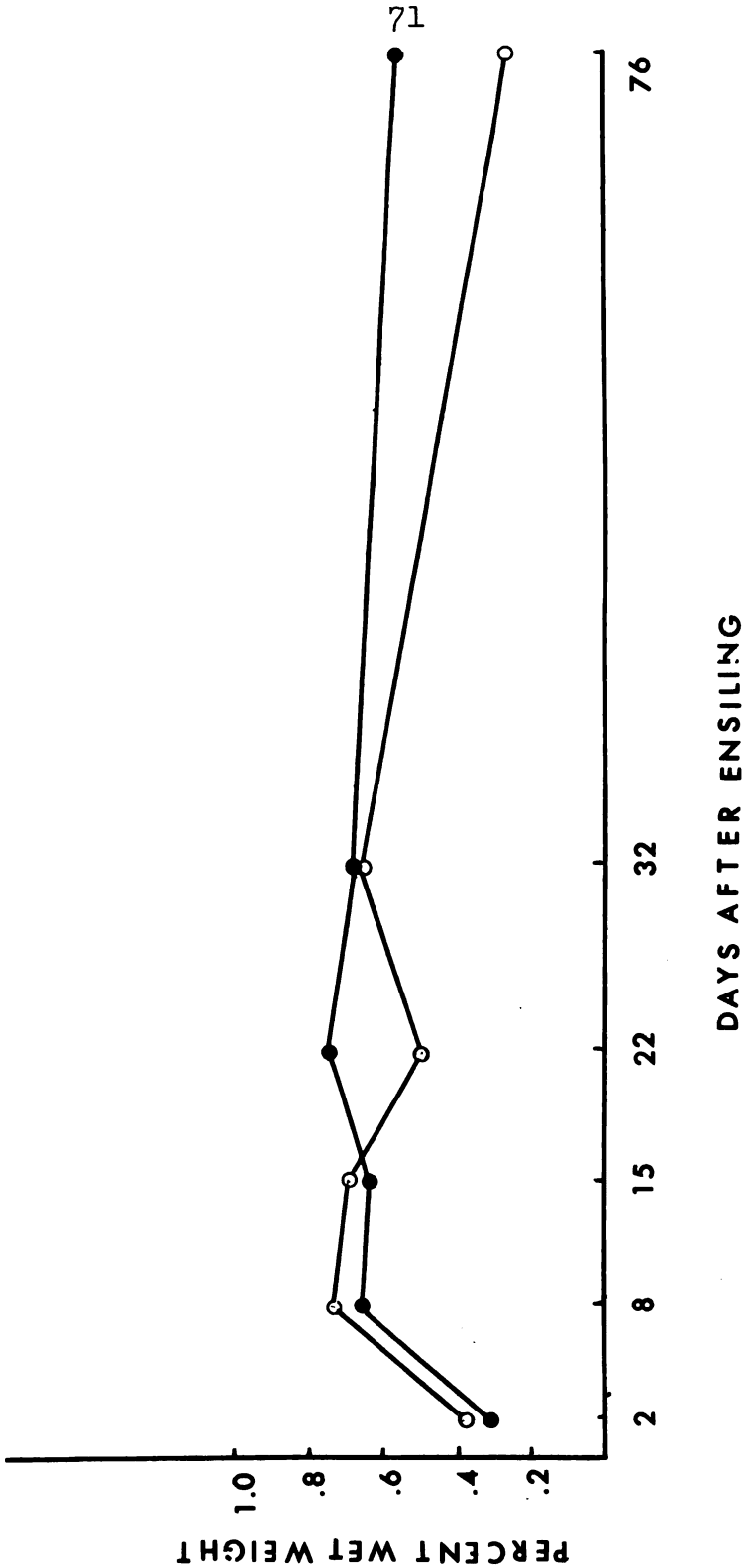

PERCENT WET WEIGHT
1.0
.8
.6
.4
.2
2
8
15
22
32
76
DAYS AFTER ENSILING

Table 19. Changes in lactic acid concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 1[a]

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Control	0.38	0.40	1.16	0.44	0.28	0.03	0.45
Propionic acid (0.5%)	0.47	0.16	1.46	0.56	0.38	0.28	0.55
Propionic acid (1.0%)	0.00	0.12	0.36	0.16	0.45	0.34	0.24
Ammonium propionate (0.5%)	0.01	0.16	0.45	0.30	0.12	0.18	0.20
Ammonium isobutyrate (0.5%)	0.36	0.24	1.29	0.59	0.14	0.21	0.47
Ammonia (0.3% nitrogen)	0.13	0.53	1.04	0.42	0.19	0.12	0.40
Paraformaldehyde (0.1%)	0.40	0.10	0.59	0.40	0.82	0.47	0.30
Formic acid to pH 3.6 + paraformaldehyde (0.1%)	0.23	0.08	0.29	0.13	0.36	0.32	0.24
K_2CO_3 (1.5%)	0.25	0.86	0.50	0.32	0.10	0.08	0.35
Foam sealants					1.26	0.86	1.06
K_2CO_3 (1.5%) + propionic acid (0.5%)	2.04	2.25	1.60	1.58	2.85	0.96	1.88
H_2SO_4 to pH 3.6	0.04	0.78	0.50	0.83	1.08	0.48	0.62
Formic acid to pH 3.6	0.04	0.76	0.04	0.42	0.20	0.49	0.32
Dried molasses (3.0%)	0.34	0.94	0.34	1.10	1.14	0.65	0.75

Table 19 (cont'd.)

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Sucrose-starch mix (3.0%)	0.29	1.27	0.29	1.00	0.94	0.36	0.69
Sodium benzoate (0.1%)	0.31	1.54	0.31	0.94	0.68	0.88	0.78
Lactobacillus casei + *L. bulgaricus*	0.32	0.95	0.32	0.68	0.46	0.30	0.50
Lactobacillus casei[b]	0.05	0.32	0.05	0.15	0.07	0.00	0.11
Wet brewers' grains without yeast[c]	0.38	0.73	0.69	0.49	0.65	0.25	0.53
Wet brewers' grains + 10% yeast[c]	0.31	0.66	0.63	0.74	0.67	0.56	0.60
Average	0.35	0.70	0.66	0.62	0.66	0.40	0.56

[a] averages of four values

[b] averages of two values

[c] averages of 66 values

in treatments with formic acid alone or with formic acid plus paraformaldehyde were similar. (6) Addition of molasses or of a sucrose-starch mixture did not produce a change in lactic acid concentration when compared to the control or among themselves. (7) Addition of sodium benzoate or of a Lactobacillus casei plus L. bulgaricus culture did not change the lactic acid content of the grains when compared to control (Table 20).

On day 32 grain treated with potassium carbonate plus propionic acid had the highest lactic acid concentration (2.85%). Grain in the buckets sealed with foam had 1.26% lactic acid. Grain to which ammonium propionate, ammonium isobutyrate, ammonia, potassium carbonate, formic acid or a lactic acid culture (L. casei) was added had lactic acid concentrations from 0.07 to 0.20%. All other treatments had lactic acid concentrations higher than the control that had 0.28% at day 32. After 76 days of ensiling, grains from all treatments, except those with L. casei culture, had higher lactic acid contents than control. The highest values were found in the grains sealed with foam (0.86%) or treated with potassium carbonate plus propionic acid (0.96%) and sodium benzoate (0.88%).

The decrease in lactic acid concentration with time may be the result of secondary fermentation in the ensiled grains.

Ammonia. The ammonia content of ensiled brewers' grains measured on days 2, 8, 15 and 22 after ensiling was variable, but increased markedly from day 8 to 15 and from day 32 to 76 (Table 21). Until day 15 and on day 32 the grains to which yeast was added had higher ammonia content than the grain

Table 20. Analysis of variance of lactic acid content of ensiled wet brewers' grains. Trial 1

Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Additives	15	2.4626	35.73**
Yeast	1	0.0104	0.15
(Additives) (Yeast)	15	0.2922	4.24**
Duplicates/trt. combination	32	0.0689	
Days	3	1.5783	28.70**
(Additives) (Days)	45	0.4873	8.86**
(Yeast) (Days)	3	0.4017	7.30**
(Additives) (Yeast) (Days)	45	0.1287	2.34**
Residual error	96	0.0550	

** Significant P<.01

Table 21. Change in ammonia concentration (mg nitrogen/100 g wet grains) with time in wet brewers' grains ensiled with different additives. Trial 1[a]

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Control	17	0	31	12	29	100	25
Propionic acid (0.5%)	40	8	18	30	32	128	35
Propionic acid (1.0%)	45	2	196	25	47	101	68
Ammonium propionate (0.5%)	24	3	140	61	29	120	61
Ammonium isobutyrate (0.5%)	50	48	69	56	65	139	65
Ammonia (0.3% nitrogen)	24	26	42	15	30	100	34
Paraformaldehyde (0.1%)	0	6	49	3	11	114	24
Formic acid to pH 3.6 + paraformaldehyde (0.1%)	7	17	12	0	8	54	14
K_2CO_3 (1.5%)	12	18	64	39	13	122	43
Foam sealants					7	80	44
K_2CO_3 (1.5%) + propionic acid (0.5%)	2	24	43	29	8	72	27
H_2SO_4 to pH 3.6	0	12	17	14	10	96	19
Formic acid to pH 3.6	27	18	12	31	34	128	34
Dried molasses (3.0%)	3	20	4	20	16	118	23

Table 21 (cont'd.)

Treatment	Day						
	2	8	15	22	32[b]	76[b]	$\bar{x}$
Sucrose-starch mix (3.0%)	6	14	0	34	14	129	25
Sodium benzoate (0.1%)	17	33	0	37	32	98	31
Lactobacillus casei + L. bulgaricus	8	16	0	24	26	107	20
Lactobacillus casei[b]	8	70	0	22	15	107	33
Wet brewers' grains without yeast[c]	12	11	33	27	20	110	36
Wet brewers grains + 10% yeast[c]	23	22	54	27	28	102	43
Average	18	17	44	27	24	106	39

[a] averages of four values

[b] averages of two values

[c] averages of 66 values

without yeast, but at day 76, grains without yeast had only lightly more ammonia than grains with yeast (Figure 8). When formic acid plus paraformaldehyde were added to the grains, the final levels of ammonia were much lower than the average for all treatments (54 mg/100 g vs 106 mg/100 g). Formic acid or paraformaldehyde alone did not decrease the levels of ammoniacal nitrogen from that of control. Addition of potassium carbonate plus propionic acid and sealing the buckets with foam resulted in ammonia levels lower than in the control after 76 days of ensiling (72 and 80 mg/100 g vs 100 mg/100 g). The highest value was found in the grains that were treated with 0.5% ammonium isobutyrate (139 mg/100 g) after 76 days of ensiling (Table 21).

Analysis of variance of the ammoniacal nitrogen content of brewers' grains from day 2 to day 22 indicated that the effects of additives, yeast and days, were significant ($P<.01$) (Table 22).

Ammonia is a product of protein hydrolysis caused by proteolytic bacteria. In this experiment only formic acid plus paraformaldehyde inhibited, although not totally, the growth of this kind of organisms and the formation of ammonia. Keeping the grains under anaerobic conditions or the presence of the different additives tested, did not result in ammonia levels lower than in the control.

Correlations among measured constituents. In this experiment wet brewers' grains stored in plastic buckets had considerable and excessive deterioration after 32 and 76 days of

Figure 8. Change in ammoniacal nitrogen content of wet brewers' grains ensiled with or without yeast. Trial 1.

(o———o) wet brewers' grains without yeast

(●———●) wet brewers' grains plus 10% yeast

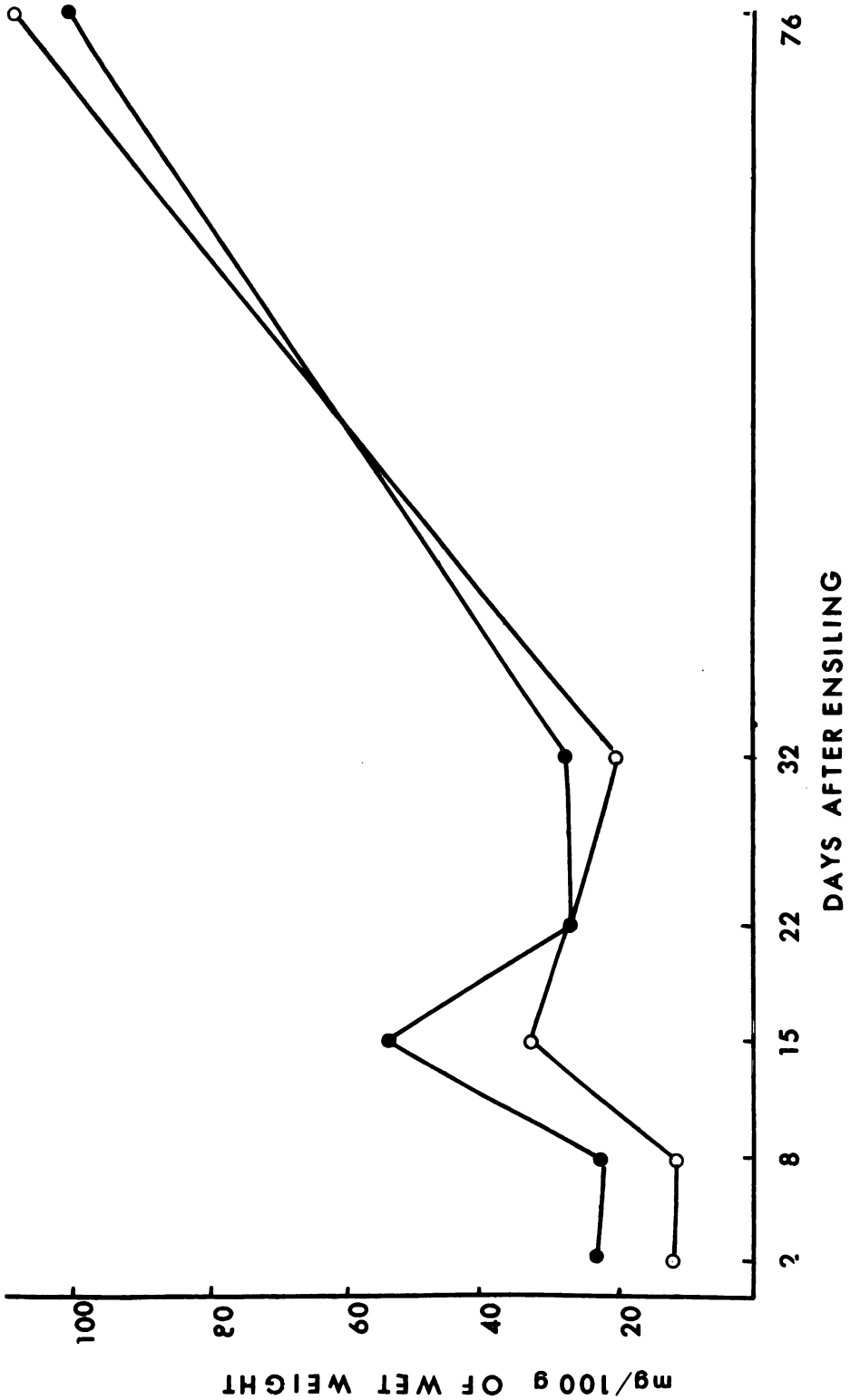

mg/100 g OF WET WEIGHT
100
80
60
40
20
2
8
15
22
32
76
DAYS AFTER ENSILING

Table 22. Analysis of variance of ammoniacal nitrogen content of ensiled brewers' grains. Trial 1

Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Additives	15	5270.75	20.46**
Yeast	1	7821.19	30.36**
(Additives) (Yeast)	15	1435.82	5.57**
Duplicates/trt. combination	32	257.57	
Days	3	9969.66	42.76**
(Additives) (Days)	45	3109.07	13.33**
(Yeast) (Days)	3	1205.14	5.17**
(Additives) (Yeast) (Days)	45	522.79	2.24**
Residual error	96	233.17	

** Significant P<.01

ensiling. Only the grains kept under anaerobic conditions had a low proportion of spoiled material. Come improvement was noticed when propionic acid, formic acid plus paraformaldehyde, a sucrose-starch mix or a lactic culture were added to the grains.

All pH values were generally low (about 4.2) but were not related to the spoilage percent (Table 24). After 32 and 76 days of ensiling the grains with the most spoilage (treated with potassium carbonate) also had the highest pH values (Table 23). Grains sealed with a cover of foam showed some spoilage in only one of the four replications and had the lowest pH. Nevertheless, other treatments that had low pH values had a high amount of spoilage.

There was a positive correlation between temperature and spoilage ($r = .68$). Aerobic fermentation increased the temperature in the silage. Range of average temperatures was rather narrow, from 76.2°F in the grains sealed with foam to 80.1°F in the grains treated with ammonium propionate.

The extent of spoilage was negatively correlated with ethanol concentration ($r = -.71$), but correlations between spoilage percentage and other measurements were not significantly different from zero (Table 24).

The pH was positively correlated to acetic acid ($r = .56$) and to butyric acid ($r = .79$) concentration. High pH values were probably favorable for the growth of microorganisms that degraded organic matter to acetic and butyric acid. Average temperature was positively related to acetic acid ($r = .49$) and to butyric acid ($r = .65$).

Table 23. Average spoilage, pH, temperature and composition of wet brewers' grains ensiled for 32 and 76 days.[a]

Treatment	% spoilage	pH	F° temperature	% DM	% protein	% ADF	% ADN	% ethanol	% acetic	% propionic	% butyric	% lactic	ammoniacal N[b] (% total N)
Control	30.5	4.15	79.8	21.7	36.0	23.3	0.91	0.28	0.64	0.30	0.45	0.15	5.10
Propionic acid (0.5%)	26.0	4.06	78.7	21.2	35.6	23.4	0.94	0.31	0.44	0.35	0.44	0.33	6.48
Propionic acid (1.0%)	37.8	3.86	79.9	22.4	36.0	23.8	0.84	0.29	0.50	1.10	0.21	0.44	5.78
Ammonium propionate (0.5%)	38.3	4.26	80.1	22.0	39.8	25.1	0.88	0.30	0.76	0.39	0.61	0.15	5.34
Ammonium isobutyrate (0.5%)	29.8	4.24	78.4	21.0	39.5	24.2	0.90	0.34	0.34	0.53	0.84	0.18	7.60
Ammonia (0.3% nitrogen)	35.0	4.08	78.8	20.4	38.5	23.8	0.91	0.24	0.46	0.24	0.68	0.16	5.18
Paraformaldehyde (0.1%)	32.8	4.25	78.4	19.5	39.4	25.3	1.13	0.16	0.31	0.17	0.72	0.64	5.22
Formic acid to pH 3.6 plus paraformaldehyde (0.1%)	24.3	3.75	76.3	23.2	35.3	23.6	1.26	0.17	0.20	0.06	0.56	0.34	2.41
Potassium carbonate (1.5%)	36.0	4.99	79.0	21.3	38.4	25.3	1.00	0.24	0.90	0.58	1.32	0.09	7.16
Foam sealants	4.5	3.86	76.2	21.7	37.2	24.5	0.99	0.65	0.24	0.19	0.46	1.06	3.18
K_2CO_3 plus propionic acid (0.5%)	38.5	4.55	78.8	21.4	36.2	22.7	0.88	0.29	0.38	0.62	0.77	1.90	3.06
Sulfuric acid to pH 3.6	34.3	3.82	77.7	19.8	39.2	22.8	0.99	0.18	0.34	0.10	0.36	0.78	4.30

Table 23 (cont'd.)

Treatment	% spoilage	pH	F° temperature	% DM	% protein	% ADF	% ADN	% ethanol	% acetic	% propionic	% butyric	% lactic	ammoniacal N[b] (% total N)
Formic acid to pH 3.6	32.3	4.18	77.5	22.9	35.9	22.0	0.86	0.28	0.34	0.12	1.12	0.56	6.03
Dried molasses (3.0%)	34.5	4.06	77.1	23.2	35.0	27.1	0.84	0.36	0.54	0.15	0.65	0.89	5.04
Sucrose-starch mix (3.0%)	23.5	4.17	77.4	22.0	37.0	23.9	0.79	0.36	0.80	0.15	0.71	0.60	5.26
Sodium benzoate (0.1%)	31.8	4.07	78.1	22.2	37.5	24.4	0.90	0.26	0.62	0.14	0.57	0.78	4.80
L. casei + L. bulgaricus	23.5	4.36	77.3	19.6	40.0	23.7	0.89	0.31	0.49	0.26	0.80	0.38	5.32
Wet brewers' grains without yeast[c]	32.0	4.28	79.8	21.1	37.8	24.9	0.97	0.23	0.63	0.44	0.68	0.45	4.90
Wet brewers' grains + 10% yeast[c]	28.9	4.06	76.6	21.7	37.2	23.2	0.89	0.30	0.36	0.20	0.60	0.62	4.98
Average	30.4	4.16	78.2	21.4	37.6	24.1	0.94	0.26	0.49	0.32	0.64	0.53	4.94

[a] averages of four values

[b] % spoilage (wet basis), DM = dry matter, % protein (dry matter basis), ADF = acid detergent fiber (dry matter basis), ADN = acid detergent insoluble nitrogen (dry matter basis), % ethanol (wet basis), % acetic acid (wet basis), % propionic acid (wet basis), % butyric acid (wet basis), % lactic acid (wet basis), ammoniacal nitrogen (% total nitrogen)

[c] averages of 68 values

Table 24. Correlation coefficients among measured constituents of ensiled wet brewers' grains. Trial 1[a]

X	Y	r[b]
spoilage	pH	0.35
	temperature	0.68**
	dry matter	-0.01
	protein	0.08
	acid detergent fiber	0.04
	acid detergent nitrogen	-0.22
	ethanol	-0.71**
	acetic acid	0.34
	propionic acid	0.39
	butyric acid	0.17
	lactic acid	-0.07
	ammoniacal nitrogen	0.31
pH	temperature	0.36
	dry matter	-0.23
	protein	0.31
	acid detergent fiber	0.16
	acid detergent nitrogen	-0.18
	ethanol	-0.13
	acetic acid	0.56*
	propionic acid	0.28
	butyric acid	0.79**
	lactic acid	-0.03
	ammoniacal nitrogen	0.44
temperature	dry matter	-0.14
	protein	0.18
	acid detergent fiber	-0.03
	acid detergent nitrogen	-0.33
	ethanol	-0.32
	acetic acid	0.49*
	propionic acid	0.65**
	butyric acid	-0.08
	lactic acid	-0.30
	ammoniacal nitrogen	0.46
dry matter	protein	-0.75**
	acid detergent fiber	0.11
	acid detergent nitrogen	-0.13
	ethanol	0.21
	acetic acid	0.11
	propionic acid	0.03
	butyric acid	-0.03
	lactic acid	0.06
	ammoniacal nitrogen	-0.17

Table 24 (cont'd.)

X	Y	r^b
protein	acid detergent fiber	0.12
	acid detergent nitrogen	0.04
	ethanol	-0.14
	acetic acid	0.13
	propionic acid	-0.03
	butyric acid	0.22
	lactic acid	-0.28
	ammoniacal nitrogen	0.29
acid detergent fiber	acid detergent nitrogen	0.04
	ethanol	0.17
	acetic acid	0.34
	propionic acid	-0.03
	butyric acid	0.08
	lactic acid	-0.12
	ammoniacal nitrogen	0.16
acid detergent nitrogen	ethanol	-0.35
	acetic acid	-0.48
	propionic acid	-0.31
	butyric acid	-0.03
	lactic acid	-0.11
	ammoniacal nitrogen	-0.40
ethanol	acetic acid	-0.05
	propionic acid	0.03
	butyric acid	-0.13
	lactic acid	0.26
	ammoniacal nitrogen	-0.10
acetic acid	propionic acid	0.21
	butyric acid	0.28
	lactic acid	-0.33
	ammoniacal nitrogen	0.44
propionic acid	butyric acid	-0.09
	lactic acid	-0.03
	ammoniacal nitrogen	0.34
butyric acid	lactic acid	-0.11
	ammoniacal nitrogen	0.40
lactic acid	ammoniacal nitrogen	-0.59*

a correlation coefficients determined on values from Table 23
b critical values for α .05 = 0.482, for α .01 = 0.606
* significantly different from 0 (P<.05)
** significantly different from 0 (P<.01)

Dry matter was negatively correlated to protein content of the dry matter (r = .75), but not to the other fractions or measurements of the silage.

The correlation coefficient between lactic acid and ammonia content of the silage was negative (r = -.59). Probably when lactic acid reached a certain concentration, unfavorable conditions existed for proteolytic bacteria growth.

Addition of autolysed yeast increased the conservation of wet brewers' grains under the conditions of this experiment. When 10% yeast was added to the grains several changes were noticed, such as 1) spoilage decreased from 32.0 to 28.9%, 2) lower pH, 3) lower storage temperatures, 4) lower values for acid detergent fiber and acid detergent insoluble nitrogen, 5) lower acetic, propionic and butyric acid concentrations, and 6) increased ethanol and lactic acid contents. Dry matter, protein and ammonia concentrations remained about the same with or without the addition of yeast.

Silage obtained in this experiment generally had high amounts of acetic, propionic and butyric acid in relation to ethanol and lactic acid contents. High amounts of ammonia indicated considerable protein breakdown. Anaerobically stored grains had very low spoilage and lactic acid content was greater than all other treatments. Yet, there was considerable acetic, propionic and butyric acid and ammonia formation in the anaerobically stored grains. Evidently the secondary fermentation that decomposes lactic acid and protein was inhibited more by storing the grains anaerobically than

with the other treatments. In general grains treated with basic compounds had more spoilage than grains with other treatments. Organic acids added to the grains decreased the proportion of spoiled material, but were not totally effective in preventing spoilage at the levels used. Neither formic acid or paraformaldehyde alone increased recovery of good silage, but when both were used together, recovery of good material was greater than for other treatments. Neither sodium benzoate or ammonium salts of propionic and isobutyric acid decreased spoilage. A sucrose-starch mix improved recovery, but dried molasses did not.

The proportion of spoiled material in the silage increased between day 32 and day 76 after ensiling. This change corresponded to an increase in pH, crude protein, acid detergent fiber, acid detergent insoluble nitrogen and ammonia content.

Crude protein content was 31.4% in the fresh grains. On day 32 the average for all treatments was 34.3% and 40.9% on day 76. This increase was probably due to a seepage of ammonia from the upper part of the grain that was spoiled and had a more extensive protein degradation.

Even when crude protein in the silage increased with time, total recovery of protein was low. Proportion of spoiled grain that was discarded was 26% on day 32 and 35% on day 76. Thus, average crude protein loss after 32 days of storage was 19% and 15% after 76 days. Ammoniacal nitrogen was 1.24% of the total nitrogen after 32 days and 4.47% after 76 days of ensiling. Considering that ammonia is a product

of protein degradation, true protein recovery in the silages was even lower than the recovery of crude protein.

Increases in acid detergent fiber and acid detergent insoluble nitrogen contents could be explained by the increase observed in temperature. Fresh brewers' grains had 0.92% acid detergent insoluble nitrogen content. This value is high compared to normal values for haylages and silages. Haylage from the middle and bottom part of a vertical silo had 0.32% acid detergent insoluble nitrogen (Thomas, 1976).

The volatile fatty acids (acetic, propionic and butyric), and ethanol in the grains increased from day 32 to day 76 and the concentration of lactic acid decreased. These phenomena indicate that a secondary fermentation occurred, in which lactic acid and other constituents were degraded to organic acids and ethanol.

In this trial the ensiled grains termed good after 76 days storage were judged to be of lower quality than those after 32 days. If ammoniacal nitrogen, lactic and butyric acid are used as indicators of silage quality, then storage longer than 32 days under conditions as in this trial are contraindicated.

Spoilage was a direct result of exposure of the grains to air. Addition of yeast improved preservation. Changes in the protein fraction of the silage should be avoided with the use of higher levels of preservatives or by maintaining anaerobic conditions during storage.

Trial 2

After studying the results of trial 1, a second experiment was carried out. Larger quantities of grain were used and the silage was kept in steel barrels instead of plastic buckets. No samples were taken from the barrels during the storage period so as not to disturb the fermentation process. Thus, in order to follow the chemical changes during ensiling, samples of fresh wet brewers' grains were put in test tubes, kept in the laboratory (about 75°F) and frozen on days 5, 7, 10 and 13 after the beginning of the experiment. Determination of the different constituents for days 32 and 60 were made on samples taken from the silage when the barrels were emptied. In all cases, the samples were taken from the portion of the material that was not spoiled.

The barrels containing the grain were exposed to ambient temperature during the months of August, September and October, to test storage conditions during warm weather. Complete preservation was achieved in trial 1 by keeping the grains under anaerobic conditions. Addition of propionic acid, formic acid plus paraformaldehyde, a sucrose-starch mix or a lactic culture reduced spoilage. These treatments or modifications were used in this experiment. Furthermore, other ways to maintain anaerobic conditions such as covering the grains with different materials were tested.

Recovery. Percentage of recovery of wet brewers' grains stored for 32 or 60 days is presented in Table 25.

Table 25. Recovery of ensiled wet brewers' grains. Trial 2

Treatment	% good after 32 days[a]	% good after 60 days[b]	$\bar{x}$
Control	82.8	67.7	75.2
Propionic acid (1%)	83.8	70.3	77.0
Propionic acid (2%)	97.5	98.0	97.8
Formic acid (1.4%) plus paraformaldehyde (0.1%)	96.2	96.7	96.4
H_2SO_4 (0.30%)	78.2	66.3	72.2
B H A (200 ppm)	73.8	63.3	68.6
Limestone on top of the grain	84.4	78.0	81.2
$CaSO_4$ on top of the grain	87.2	80.7	84.0
Dry molasses on top of the grain	79.0	65.7	72.4
Liquid molasses on top of the grain	87.8	79.3	83.6
Liquid molasses (7%)	73.8	76.3	75.0
Ground corn on top of the grain	80.4	72.7	76.6
Ground corn (10%)	80.5	71.7	76.1
Lactic acid culture	73.2	65.0	69.1

Table 25 (cont'd.)

Treatment	% good after 32 days [a]	% good after 60 days [b]	$\bar{x}$
Lactic culture + molasses (7%)	76.8	65.7	71.2
Lactic culture + ground corn (10%)	83.3	66.0	74.6
Sealed with a plastic bag full of water on top	97.0	100.0	98.5
Average	83.8	75.5	79.4

[a] average of two values

[b] one value

Six days after the beginning of the experiment spoilage was noticed in the grains treated with BHA, 10% ground corn and in the control with no additive. Small mold colonies were seen on the surface of the grains covered with limestone or dry molasses.

After 11 days, only the grains treated with propionic, formic and sulfuric acid had not developed surface spoilage. The grains covered with molasses, calcium sulfate and ground corn smelled rotten. Flies started to grow beneath the surface of the grains with no additive and of the grain with BHA, 7% molasses, 10% ground corn and lactic acid culture. Grains treated with propionic acid (1%) and sulfuric acid started to have surface spoilage after 15 days of ensiling. At this time about one centimeter of the surface of the grain had dried in most of the treatments while spoilage continued to develop underneath. Dry limestone put on top of the grain (about 1 cm thick) absorbed water from the grains and remained wet until day 22 after the beginning of the experiment. Calcium sulfate was layered on top of the grains as a slurry and remained soft until day 11, then started to dry and harden. After 22 days the calcium sulfate layer was hard and started to crack especially on the edges, then flies grew beneath the cracked surface.

Two of the three barrels of each treatment were emptied after 32 days of ensiling, and the remaining one after 60 days. Recovery of good silage and quantity of spoilage were measured.

Grain from the barrels that had a plastic bag filled with water on top did not have any spoilage and the silage

looked and smelled fresh. Weight of the water in the plastic bag formed a seal between the grain and the environment keeping the grain entirely anaerobic. Grain treated with propionic acid (2%) and with formic acid plus paraformaldehyde had a dry surface and no spoilage after 32 or 60 days of ensiling. Silage from these two treatments had an acid smell and a darker color than others. Grain treated with sulfuric acid was darkest of all. All other treatments had more or less surface spoilage and fly maggots growing thereon. When the spoiled material was separated and discarded all remaining portion looked and smelled about the same from all treatments, except those treated with acids.

In all cases the spoiled material had a dark brown color, an offensive-rotten smell and fly maggots growing in it. Covering the surface with limestone, calcium sulfate, dried or liquid molasses, and ground corn did not prevent spoilage or the growth of flies.

Plastic bags lining the barrels allowed no seepage from the storage container.

Grains from the treatments covered with limestone, calcium sulfate or liquid molasses had less spoilage than did control. Covering the surface with dried molasses or ground corn did not have any effect on the recovery when compared to the control. Addition of a lactic acid culture did not improve storability. Liquid molasses or ground corn mixed with the grain did not have any effect on recovery. Propionic acid at a level of 1% allowed more spoilage than did 2%

propionic acid. Acidification of the mass with sulfuric acid did not increase recovery of good material. Treating the grain with butylated hydroxyanisol (BHA) decreased percentage recovery. This additive probably did not have any effect on the fermentation, but the initial mixing allowed more contact with air which may have increased spoilage.

After 32 days of ensiling the spoilage for all treatments averaged about 17%, and after 60 days spoilage increased to about 25%. After 60 days the grains with no additive, with sulfuric acid, BHA, dry molasses on top of the grain and those with a lactic culture, had about 35% spoilage. Grains treated with propionic acid (1%), ground corn on top or mixed with ground corn had an average of 30% spoilage while those covered with limestone, calcium sulfate or dry molasses had only 20% spoilage.

Effectiveness in preventing spoilage was due either to airtight sealing that did not allow aerobic fermentation to occur, or to the presence of sufficient concentration of preservatives such as propionic or formic acid mixed with paraformaldehyde. Acidity per se was not a factor in preventing spoilage. Addition of sulfuric acid did not have a beneficial effect. Good conservation of brewers' grains silage was the result of preservative qualities of propionic acid, formic acid and paraformaldehyde.

pH. Fresh brewers' grains had a pH of 5.3. This value decreased markedly at five days of ensiling when the treatments had an average of 3.98 (Table 26).

Table 26. Changes in pH with time in wet brewers' grains ensiled with different additives. Trial 2

Treatment	Day						$\bar{x}$
	5[a]	7[a]	10[a]	13[a]	32[b]	60[b]	
Control	3.90	4.00	4.05	4.25	3.62	3.65	3.91
Propionic acid (1%)	4.00	4.10	5.30	5.60	3.68	3.90	4.43
Propionic acid (2%)	3.95	4.00	4.20	4.20	3.98	4.00	4.06
Formic acid (1.4%) + paraformaldehyde (0.1%)	3.10	3.15	3.20	3.20	3.25	3.30	3.20
Sulfuric acid (0.3%)	2.90	2.80	3.05	2.90	2.83	3.00	2.91
B H A (200 ppm)	3.85	3.60	3.35	3.75	3.58	3.60	3.62
Liquid molasses (7%)	4.40	3.70	3.95	3.90	3.78	3.75	3.91
Ground corn (10%)	4.35	3.80	5.55	5.80	3.60	3.65	4.46
Lactic acid culture	4.15	4.00	4.50	4.20	3.72	3.70	4.04
Lactic culture + molasses (7%)	4.25	3.90	4.10	3.90	3.69	3.75	3.93
Lactic culture + ground corn (10%)	4.95	3.60	5.70	5.60	3.78	3.75	4.56
Sealed	4.00	3.80	4.00	3.90	3.72	3.50	3.82
Average	3.98	3.70	4.24	4.27	3.60	3.63	3.91

[a] values determined on samples kept in test tubes

[b] values determined on samples taken from two barrels on days 32 and one barrel on day 60

On days 10 and 13 grains treated with propionic acid (1%), 10% ground corn or lactic acid culture had pH values above 5.3, while all others were below 4.25. All other treatments had a stable or decreasing pH value during the ensiling process. All samples taken from silages kept for 32 or 60 days in barrels had pH values of 4.0 or lower.

Grains with formic or sulfuric acid added had the lowest pH values during the ensiling period. On day 60 the values were 3.30 and 3.00, respectively. At this time the grains treated with 1 or 2% propionic acid had a higher pH value than did control (3.9 and 4.0 vs 3.6).

Addition of molasses or ground corn did not produce a silage having a pH lower than control.

The pH of the grains to which a lactic culture was added were not lower than pH of similar treatments without the culture at day 32 or day 60.

The pH values indicate that an active fermentation occurred during the early days of the ensiling process. Acid addition initially lowered pH of the grains, but with other treatments low pH values occurred as a result of the fermentation process.

Temperature. The brewers' grains had a temperature of 140°F arrived by truck from the brewery. Thermocouple temperatures taken during storage are presented in Table 27. Ambient temperatures taken in the area where barrels were located had an average of 79°F during the time when the experiment was performed.

Table 27. Changes in temperature (F) with time in wet brewers' grains stored with different additives or methods of ensiling. Trial 2[a]

Treatment	Day												$\bar{x}$
	2	5	6	8	11	13	15	19	23	27	29	32	
Control	88.0	78.3	78.0	80.0	82.7	80.0	79.7	71.3	77.0	73.0	73.7	71.3	77.8
Propionic acid (1%)	85.3	70.3	72.3	74.7	77.3	76.0	80.7	77.0	79.7	77.3	82.0	68.3	76.8
Propionic acid (2%)	86.7	69.3	69.3	73.0	75.3	74.0	78.7	67.3	70.7	69.3	72.0	66.0	72.6
Formic acid (1.4%) + paraformaldehyde (0.1%)	85.3	70.7	70.7	70.3	76.0	74.0	73.7	68.0	69.7	69.7	71.3	70.3	72.5
H_2SO_4 (0.3%)	88.7	70.7	69.7	71.0	77.7	76.7	77.7	71.3	73.3	76.0	75.0	74.3	75.2
B H A (200 ppm)	95.0	76.0	73.0	78.0	81.7	80.7	80.0	73.0	74.7	74.3	78.3	74.7	78.3
Limestone on top of the grain	98.3	70.3	69.7	70.0	74.0	74.0	73.7	69.0	64.7	67.7	72.7	71.3	72.5
$CaSO_4$ on top of the grain	94.7	68.0	70.0	70.7	74.7	73.0	74.7	68.7	72.3	70.3	72.7	68.0	73.1
Dry molasses on top of the grain	96.7	71.0	71.3	72.3	78.7	77.7	76.0	71.7	73.0	73.0	73.3	67.7	75.2
Liquid molasses on top of the grain	98.3	77.0	69.0	70.7	75.7	71.3	72.3	66.0	77.0	70.7	72.3	71.7	74.3
Liquid molasses (7%)	90.0	69.3	72.3	75.3	79.0	76.0	75.3	71.3	64.3	71.7	72.7	72.7	74.2

Table 27 (cont'd.)

Treatment	Day												$\bar{x}$
	2	5	6	8	11	13	15	19	23	27	29	32	
Ground corn on top of the grain	98.0	73.0	73.0	74.0	78.3	74.0	73.7	66.7	71.3	70.3	72.3	71.0	74.7
Ground corn (10%)	91.7	76.0	76.7	75.7	81.3	79.0	79.7	74.3	76.7	77.3	79.7	78.3	78.7
Lactic acid culture	95.7	71.7	74.3	75.3	76.7	76.7	77.3	70.7	72.3	70.7	76.7	77.0	76.2
Lactic culture + molasses (7%)	95.3	71.7	79.0	79.0	81.3	87.7	82.3	78.0	76.7	76.3	79.7	67.0	79.0
Lactic culture + ground corn (10%)	96.0	75.7	76.0	74.7	78.3	78.0	76.3	72.7	75.0	75.3	76.3	73.0	77.2
Sealed with a plastic bag full of water on the top	96.3	69.7	69.3	69.3	73.7	70.7	71.0	68.0	67.3	68.0	69.3	68.0	71.7
Average	92.6	72.2	72.7	73.7	77.7	76.4	76.6	70.7	72.7	72.4	74.7	71.2	75.3
Ambient temperature	80.0	80.0	80.0	86.0	81.0	80.0	89.0	72.0	79.0	87.0	62.0	74.0	79.2

[a] averages of four values

The second day after ensiling, temperature of the stored grain was above ambient. By the fifth day, temperatures had decreased below ambient. Temperatures generally remained below ambient except for day 29. From day 11 to 15 temperatures of the silage were higher than for other days, corresponding to a higher ambient temperature. Temperatures were taken about midday and internal temperature may also have been affected by ambient temperature hours previous to reading.

Temperatures among treatments were significantly different ($P<.01$, Table 28).

During the experimental period, average temperature of the control, no additive, was higher than average temperature of the treatment sealed with a plastic bag full of water (77.8 vs 71.7, $P<.10$). Temperature for treatments with 2% propionic acid, formic acid plus paraformaldehyde, limestone on top of the grain and calcium sulfate on top of the grain were not significantly different from the treatment covered with a plastic bag full of water. The latter had the lowest temperature of all treatments. Temperatures of treatments with propionic acid (1%), sulfuric acid, dried or liquid molasses on top of the grain were statistically similar. Temperatures of the treatments with BHA, lactic acid culture plus 10% molasses and lactic acid culture plus 10% corn did not differ significantly from control when individual contrasts were made. Grains covered with limestone, calcium sulfate, dried or liquid molasses, had lower average

Table 28. Analysis of variance of temperatures of ensiled wet brewers' grains. Trial 2

Source of Variance	Degrees of Freedom	Mean Square	F Statistic
Treatments	16	214.0537	2.61**
Barrels/treatment	34	81.8693	
Days	11	1764.6683	185.44**
(Treatments) (Days)	176	21.7014	2.28**
Residual error	374	9.5163	

temperatures than uncovered grains without additives (73.8 vs 77.8), but the differences were not significant.

Temperatures appeared to be related to the degree of anaerobiosis in the stored grains.

Dry matter and protein. Average dry matter of the silage was 22.5% at day 32 and 22.2% at day 60 (Table 29). Dry matter content of the grains was increased up to 25 and 27% by the addition of liquid molasses or ground corn. Dry matter content for other treatments were similar to control, and similar for covered and open barrels.

Liquid was observed draining from the truck in which the grains came from the brewery, but during the experiment no moisture was lost since the barrels were lined with impermeable polyethylene plastic bags. Sample for dry matter was taken from several places in the central portion of the partially mixed contents and would not reflect changes near the surface. Protein content of fresh brewers grains was 31.4% (dry matter basis) and had only slight changes during the ensiling process (Table 29).

Addition of 10% ground corn or 7% liquid molasses decreased the protein concentration of the grains as expected. Apart from these treatments protein ranged from 35.4% for the treatment with lactic culture to 31.0% for the treatment with formic acid plus paraformaldehyde.

When the barrels were emptied and the spoiled material discarted, no bad smell was noticed from the silage, thus, protein decomposition was nil. On the other hand, the spoiled

Table 29. Dry matter and protein content of ensiled wet brewers' grains. Trial 2[a]

Treatment	% dry matter			% protein (dry matter basis)		
	after 32 days	after 60 days	$\bar{x}$	after 32 days	after 60 days	$\bar{x}$
Control	21.3	19.3	20.3	33.7	33.4	33.6
Propionic acid (1%)	19.1	20.1	19.6	35.2	34.8	35.0
Propionic acid (2%)	20.0	22.5	21.2	34.7	30.6	32.6
Formic acid (1.4%) + paraformaldehyde (0.1%)	23.2	22.9	23.0	32.8	31.1	32.0
H_2SO_4 (0.3%)	22.2	22.6	22.4	33.0	29.1	31.0
B H A (200 ppm)	22.3	20.2	21.2	33.1	34.7	33.9
Limestone on top of the grain	20.6	21.4	21.0	35.6	33.8	34.7
$CaSO_4$ on top of the grain	22.1	21.4	21.8	34.1	35.6	34.8
Dry molasses on top of the grain	23.2	21.2	22.2	30.6	32.6	31.6
Liquid molasses on top of the grain	22.3	22.1	22.2	35.2	34.5	34.8
Liquid molasses (7%)	25.5	23.3	24.4	29.1	30.0	29.6
Ground corn on top of the grain	21.5	22.3	21.9	35.8	30.9	33.4

Table 29 (cont'd.)

Treatment	% dry matter			% protein (dry matter basis)		
	after 32 days	after 60 days	$\bar{x}$	after 32 days	after 60 days	$\bar{x}$
Ground corn (10%)	27.0	27.2	27.1	26.6	25.7	26.2
Lactic acid culture	22.2	21.5	21.8	36.5	34.4	35.4
Lactic culture + molasses (7%)	22.4	24.5	23.4	33.9	30.6	32.2
Lactic culture + ground corn (10%)	26.4	24.5	25.4	28.4	30.2	29.3
Sealed with a plastic bag full of water on the top	21.6	20.8	21.2	32.7	36.9	34.8
Average	22.5	22.2	22.4	33.0	32.3	32.6

[a] values from two barrels on day 32 and one barrel on day 60
composition of the grains as received was 24.2% ADF and 0.92 ADN (dry matter basis)

material had a strong rotten smell, a result of an active microbial degradation of the grain's organic matter.

These results indicate no loss of nitrogen when brewers' grains are ensiled as in these trials.

Acid detergent fiber and acid detergent insoluble nitrogen. Acid detergent fiber of fresh brewers' grains was 24.2% (dry matter basis) and the average did not change during the ensiling process (Table 30).

Grain to which liquid molasses or ground corn was added had lower acid detergent fiber than did other treatments due to dilution with low fiber addition. Addition of formic acid plus paraformaldehyde increased the fiber content of the grain after 32 days of ensiling, but after 60 days those values were similar to those of other treatments.

Average acid detergent insoluble nitrogen (ADN) content of the grain was the same at day 32 and day 60 (0.87%). The percent in fresh brewers' grains was 0.92 (18% of the total nitrogen). Mixing of liquid molasses or ground corn with the grain decreased its ADN content. High values were observed for the control (1.02%) and for the grains covered with liquid molasses (0.95%) in relation to other treatments. Values for other treatments ranged from 0.82 to 0.90%. This corresponds to about 16% of the total nitrogen.

ADN of the grains treated with formic acid plus paraformaldehyde was greater than any other treatment. This value was about 40% above all others and amounted to 24% of the total nitrogen. Formaldehyde binds proteins and must

Table 30. Acid Detergent Fiber (ADF) and Acid Detergent insoluble Nitrogen (ADN) content of ensiled wet brewers' grains. Trial 2[a]

Treatment	% ADF (% DM)			% ADN (% DM)		
	after 32 days	after 60 days	$\bar{x}$	after 32 days	after 60 days	$\bar{x}$
Control	25.2	25.1	25.2	1.00	1.03	1.02
Propionic acid (1%)	24.0	24.9	24.4	0.86	0.92	0.89
Propionic acid (2%)	25.2	25.4	25.3	0.87	0.86	0.86
Formic acid (1.4%) + paraformaldehyde (0.1%)	27.0	25.0	26.0	1.25	1.19	1.22
H_2SO_4 (0.3%)	24.1	22.0	23.0	0.93	0.83	0.88
B H A (200 ppm)	24.7	24.7	24.7	0.88	0.90	0.89
Limestone on top of the grain	24.7	23.4	24.0	0.93	0.88	0.90
$CaSO_4$ on top of the grain	25.2	24.7	25.0	0.88	0.88	0.88
Dry molasses on top of the grain	23.2	25.9	24.6	0.83	0.84	0.84
Liquid molasses on top of the grain	24.4	24.7	24.6	0.88	1.02	0.95
Liquid molasses (7%)	21.8	19.8	20.4	0.77	0.76	0.76

Table 30 (cont'd.)

Treatment	% ADF (% DM) after 32 days	% ADF (% DM) after 60 days	$\bar{x}$	% ADN (% DM) after 32 days	% ADN (% DM) after 60 days	$\bar{x}$
Ground corn on top of the grain	24.8	25.3	25.0	0.82	0.82	0.82
Ground corn (10%)	19.0	19.1	19.1	0.64	0.60	0.62
Lactic acid culture	24.8	24.9	24.8	0.86	0.85	0.86
Lactic culture + molasses (7%)	21.9	22.7	22.3	0.79	0.73	0.76
Lactic culture + ground corn (10%)	20.8	19.9	20.4	0.75	0.78	0.76
Sealed with a plastic bag full of water on the top	24.7	25.1	24.9	0.84	0.86	0.85
Average	23.9	23.7	23.8	0.87	0.87	0.87

[a] values from two barrels on day 32 and one barrel on day 60
composition of the grains as received was 24.2% ADF and 0.92 ADN (dry matter basis)

make them insoluble in acid detergent solution.

For comparison, ADN in fresh forage amounts to 5-9% of total nitrogen and increases with extent of heating during ensiling (Yu and Thomas, 1976). Regression equations have been developed to calculate nitrogen digestibility from ADN values in forages (Goering et al., 1973; Yu and Thomas, 1976).

Ethanol. Ethanol content of the grains increased during the ensiling process, but the rate of increase was not the same for all treatments (Table 31).

Grains treated with acids (propionic, formic and sulfuric) contained little ethanol. Propionic acid at 2% had a greater effect than did propionic acid at 1%.

Grains to which a lactic acid culture plus 7% molasses were added had a high ethanol content (over 2%) throughout (day 5 to 60), while the treatment with 7% liquid molasses alone had only 0.05% ethanol on day 5 which increased to 1.74% on day 60. On day 32 and 60 silage from the treatment with lactic acid culture plus 10% corn had next to the highest of any treatment (1.54 to 2.07%). Some of the ground corn mixed with the grain could still be observed in the silage at the end of the experiment. Molasses, being a more soluble carbohydrate source, was probably degraded faster and more completely than ground corn by the microbiota during the fermentation process. Treatment with a lactic acid culture plus a substrate resulted in higher ethanol concentrations than treatment with the culture or the substrate alone.

Table 31. Changes in ethanol concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 2

Treatment	Day						
	5[a]	7[a]	10[a]	13[a]	32[b]	60[b]	$\bar{x}$
Control	0.10	0.14	0.32	0.22	1.17	1.04	0.49
Propionic acid (1%)	0.29	0.08	0.00	0.00	0.53	0.54	0.24
Propionic acid (2%)	0.01	0.02	0.00	0.02	0.26	0.20	0.08
Formic acid (1.4%) + paraformaldehyde (0.1%)	0.01	0.02	0.00	0.02	0.03	0.02	0.02
Sulfuric acid (0.3%)	0.05	0.00	0.00	0.02	0.08	0.06	0.04
B H A (200 ppm)	0.14	0.07	0.07	0.34	1.35	1.51	0.58
Liquid molasses (7%)	0.05	0.27	0.32	0.36	1.58	1.74	0.72
Ground corn (10%)	0.34	2.28	0.21	0.02	0.58	1.65	0.85
Lactic acid culture	0.42	1.03	0.34	1.11	1.49	0.67	0.84
Lactic culture + molasses (7%)	2.45	2.52	1.81	2.47	2.14	3.18	2.42
Lactic culture + ground corn (10%)	0.37	2.51	0.01	1.01	1.54	2.07	1.08
Sealed	0.35	2.02	2.24	0.82	1.52	1.17	1.35
Average	0.38	0.91	0.44	0.45	1.02	1.15	0.73

[a] values determined on samples kept in test tubes

[b] values determined on samples taken from two barrels on day 32 and one barrel on day 60 ethanol concentration in the grains as received was 0.01% (on a wet basis)

Addition of the lactic acid culture alone increased average ethanol from 0.49 to 0.84%.

Source of the microbiota, presumably yeasts, that form ethanol is speculative but probably originated at the brewery, delivery truck or other sources.

Acetic acid. Acetic acid content in fresh brewers' grains was 0.07% (on a wet basis). This concentration did not change during the ensiling process in control, in the grains with 2% propionic acid, sulfuric acid, 10% ground corn, and in the treatment sealed with a plastic bag full of water (Table 32).

At day 32 and 60 grains from treatments with lactic acid culture and with 7% liquid molasses had a higher acetic acid content than did other treatments. Concentrations of acetic acid in butylated hydroxyanisol (BHA) treated grains started to increase on day 10, but in other treatments the increase started sooner. Grain treated with formic acid plus paraformaldehyde had more acetic acid than did grain with propionic acid 2%, sulfuric acid and the control.

Addition of liquid molasses of ground corn combined with the lactic acid culture had more effect on acetic acid formation than ground corn, molasses or lactic acid culture individually. Acetic acid in the silage could be the product of heterolactic fermentation or the result of secondary fermentation of ethanol or lactic acid.

Propionic acid. Fresh brewers' grains did not have any measurable amount of propionic acid. Propionic acid content

Table 32. Changes in acetic acid concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 2

Treatment	Day						
	5[a]	7[a]	10[a]	13[a]	32[b]	60[b]	$\bar{x}$
Control	0.07	0.12	0.08	0.09	0.07	0.09	0.09
Propionic acid (1%)	0.08	0.34	0.02	0.03	0.31	0.17	0.16
Propionic acid (2%)	0.05	0.05	0.04	0.06	0.14	0.10	0.07
Formic acid (1.4%) + paraformaldehyde (0.1%)	0.13	0.12	0.14	0.16	0.14	0.12	0.14
Sulfuric acid (0.3%)	0.05	0.09	0.03	0.05	0.10	0.11	0.07
B H A (200 ppm)	0.08	0.05	0.12	0.73	0.14	0.15	0.21
Liquid molasses (7%)	0.12	0.36	1.43	0.80	0.32	0.35	0.56
Ground corn (10%)	0.10	0.16	0.15	0.06	0.10	0.11	0.11
Lactic acid culture	0.12	0.10	0.06	0.15	0.22	0.29	0.16
Lactic culture + molasses (7%)	0.18	0.17	0.16	0.25	0.27	0.35	0.23
Lactic culture + ground corn (10%)	0.13	0.10	0.05	0.05	0.28	0.42	0.17
Sealed	0.10	0.11	0.10	0.15	0.06	0.09	0.10
Average	0.10	0.15	0.20	0.22	0.18	0.20	0.17

[a] values determined on samples kept in test tubes

[b] values determined on samples taken from two barrels on day 32 and one barrel on day 60 acetic acid concentration in the grains as received was 0.07% (on a wet basis)

increased during the ensiling process, but the final concentration in the silage was very low in relation to the other acids or to ethanol (Table 33).

Grain treated with propionic acid kept its concentration constant during 60 days of storage. The actual concentration of propionic acid on day 32 and 60 in silage treated with this preservative was 1.56 and 3.00%.

Butyric acid. Butyric acid was not measurable in most of the treatments during or after the ensiling process, when the spoiled material was separated from the good silage (Table 34). This fact indicates that under the conditions of this experiment clostridial activity was low.

Lactic acid. Lactic acid content of fresh brewers' grains was 0.14%. Lactic acid formation was very evident by day 5 in most of the treatments, and its concentration increased with time from 0.65% on day 5 up to 1.84% on day 60 (Table 35).

Addition of propionic acid, formic acid plus paraformaldehyde and sulfuric acid inhibited lactic acid formation. Propionic acid at a level of 2% was more effective in preventing lactic acid formation than was 1% propionic acid. Inhibition was most marked with formic acid plus paraformaldehyde or with sulfuric acid. This indicates reduced microbial fermentation in these acid treatments.

When a source of carbohydrates was added to the grain, lactic acid content of the silage was increased. Addition of molasses resulted in a higher lactic acid content than

Table 33. Changes in propionic acid concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 2

Treatment	Day						
	5[a]	7[a]	10[a]	13[a]	32[b]	60[b]	$\bar{x}$
Control	0.00	0.01	0.03	0.01	0.01	0.02	0.01
Propionic acid (1%)	1.62	1.98	1.90	1.86	1.67	1.47	1.75
Propionic acid (2%)	2.98	3.08	2.99	2.94	2.90	3.09	3.00
Formic acid (1.4%) + paraformaldehyde (0.1%)	0.00	0.01	0.04	0.01	0.05	0.02	0.02
Sulfuric acid (0.3%)	0.00	0.01	0.01	0.01	0.00	0.04	0.01
B H A (200 ppm)	0.00	0.02	0.03	0.00	0.03	0.03	0.02
Liquid molasses (7%)	0.00	0.01	0.01	0.01	0.02	0.03	0.01
Ground corn (10%)	0.00	0.01	0.01	0.04	0.03	0.01	0.02
Lactic acid culture	0.00	0.01	0.01	0.01	0.02	0.04	0.02
Lactic culture + molasses (7%)	0.00	0.01	0.01	0.01	0.02	0.02	0.01
Lactic culture + ground corn (10%)	0.00	0.01	0.02	0.01	0.03	0.05	0.02
Sealed	0.00	0.01	0.00	0.01	0.02	0.03	0.01
Average	0.38	0.43	0.42	0.41	0.40	0.40	0.41

[a] values determined on samples kept in test tubes

[b] values determined on samples taken from two barrels on day 32 and one barrel on day 60 propionic acid concentration in the grains as received was nil

Table 34. Changes in butyric acid concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 2

Treatment	Day						
	5[a]	7[a]	10[a]	13[a]	32[b]	60[b]	$\bar{x}$
Control	0.00	0.00	0.00	0.05	0.02	0.03	0.01
Propionic acid (1%)	0.01	0.02	0.00	0.00	0.02	0.03	0.01
Propionic acid (2%)	0.01	0.02	0.01	0.03	0.02	0.02	0.02
Formic acid (1.4%) + paraformaldehyde (0.1%)	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Sulfuric acid (0.3%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B H A (200 ppm)	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Liquid molasses (7%)	0.00	0.00	0.03	0.03	0.00	0.00	0.01
Ground corn (10%)	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Lactic acid culture	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lactic culture + molasses (7%)	0.00	0.00	0.04	0.03	0.01	0.02	0.02
Lactic culture + ground corn (10%)	0.00	0.00	0.00	0.02	0.00	0.00	0.00
Sealed	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Average	0.00	0.00	0.01	0.01	0.01	0.01	0.01

[a] values determined on samples kept in test tubes

[b] values determined on samples taken from two barrels on day 32 and one barrel on day 60 butyric acid concentration in the grains as received was nil

Table 35. Changes in lactic acid concentration (percent on a wet basis) with time in wet brewers' grains ensiled with different additives. Trial 2

Treatment	Day						
	5[a]	7[a]	10[a]	13[a]	32[b]	60[b]	$\bar{x}$
Control	1.17	1.34	1.54	1.55	2.30	2.72	1.77
Propionic acid (1%)	0.95	0.36	0.00	0.00	1.60	1.88	0.80
Propionic acid (2%)	0.00	0.12	0.05	0.00	0.76	0.88	0.30
Formic acid (1.40%) + paraformaldehyde (0.1%)	0.00	0.00	0.02	0.09	0.24	0.34	0.12
Sulfuric acid (0.3%)	0.00	0.00	0.01	0.00	0.20	0.33	0.09
B H A (200 ppm)	0.90	1.25	1.65	1.47	2.27	2.32	1.64
Liquid molasses (7%)	0.90	1.46	2.43	1.77	2.81	2.72	2.02
Ground corn (10%)	0.53	1.43	0.07	0.09	2.17	2.11	1.07
Lactic acid culture	0.66	0.79	0.64	0.83	1.56	1.65	1.02
Lactic culture + molasses (7%)	1.40	1.40	1.03	1.98	2.12	2.38	1.72
Lactic culture + ground corn (10%)	0.04	1.40	0.00	0.21	1.59	2.52	0.96
Sealed	1.06	1.40	1.18	1.18	1.54	2.21	1.43
Average	0.63	0.91	0.72	0.76	1.60	1.84	1.08

[a] values determined on samples kept in test tubes

[b] values determined on samples taken from two barrels on day 32 and one barrel on day 60 lactic acid concentration in the grain as received was 0.14%

the addition of corn. The use of a lactic starter, alone or in combination with the addition of a substrate, did not result in an increase in lactic acid concentration of the final product. On day 32 and 60 the control had higher concentrations of lactic acid (2.30 and 2.72%) than any other treatment, except that with 7% molasses (2.81 and 2.72%).

In general, none of the treatments increased lactic acid concentration above that of control. Marked inhibition of lactic acid formation was due to the addition of acids.

Ammonia nitrogen. Ammonia nitrogen was not detected in fresh brewers' grains. The pattern of ammonia formation during the ensiling process was irregular but levels in all treatments were generally low.

Grains from several treatments had little or no ammonia on day 30 or 60. Treatments allowing little ammonia formation were control, propionic acid, corn mixed and plastic bag on top. The highest value was 50 mg/100 g for the grain without additive after 13 days storage in test tubes (Table 36).

Addition of liquid molasses, with or without a lactic culture resulted in ammonia levels higher than in other treatments on day 32 or 60. No ammonia was found on day 5. Higher ammonia concentration were found in days 7, 10 and 13 of test tubes storage than after 32 or 60 days of barrel storage. Evidently there is much more proteolysis in small quantities of grains stored in test tubes than larger quantities stored in barrels (Table 36).

Table 36. Changes in ammonia concentration (mg nitrogen/100 g wet grain) with time in wet brewers' grains ensiled with different additives. Trial 2

	Day						
	5[a]	7[a]	10[a]	13[a]	32[b]	60[b]	$\bar{x}$
Control	0	1	35	50	0	1	14
Propionic acid (1%)	0	1	8	0	0	0	2
Propionic acid (2%)	0	1	3	0	0	0	1
Formic acid (1.4%) + paraformaldehyde (0.1%)	0	0	16	0	2	0	3
Sulfuric acid (0.3%)	0	3	31	9	9	2	9
B H A (200 ppm)	0	5	21	12	2	0	7
Liquid molasses (7%)	0	6	0	13	15	33	11
Ground corn (10%)	0	23	2	0	0	0	4
Lactic acid culture	0	31	3	16	2	3	9
Lactic culture + molasses (7%)	0	27	0	16	6	20	12
Lactic culture + ground corn (10%)	0	29	1	0	5	0	6
Sealed	0	5	26	0	0	0	5
Average	0	11	12	10	3	5	7

[a] values determined on samples kept in test tubes

[b] values determined on samples taken from two barrels on day 32 and one barrel on day 60 ammonia concentration in the grain as received was nil

Low ammonia levels in the silage are an indication that protein degradation was not extensive in the unspoiled portion of the silage.

Microbiological examination. Estimates of microbiological numbers for grain samples on day 60 are presented in Table 37.

Total counts of microorganisms range from a high of 4.0×10^6 cells/g in grains treated with dry molasses on top to a low of 1.8×10^5 cells/g in the treatment with calcium sulfate on top. Propionic acid did not have an inhibitory effect on the number of bacteria in grains on day 60, however, formic acid plus paraformaldehyde decreased the microbial population almost ten times when compared to the control. Treatments with sulfuric acid, calcium sulfate on top of the grain, mixed liquid molasses, ground corn on top and mixed with the grains, and the treatment sealed with a plastic bag full of water, had less microorganisms than did control. Treatments with propionic acid, butylated hydroxyanisol (BHA), dried and liquid molasses on top of the grain had numbers of microorganisms that were close or higher than the control.

Determination of coliforms was made due to the fact that the experiment was performed near dairy barns, and the original grains were dumped on the floor of a bunker silo before ensiling. No coliform organisms were detected at the end of the ensiling period, but they may have been present at ensiling time. Usually these microorganisms are not present at the end of anaerobic microbial fermentations with mixed flora (Weise, 1969).

Table 37. Microbiological tests on wet brewers' grains ensiled during 60 days (microorganisms per gram of ensiled wet brewers' grains). Trial 2

Treatment	Total count	Coliforms	Lactobacilli	Yeasts and molds
Control	2.3×10^6	no growth	no growth	7.2×10^4
Propionic acid (1%)	1.5×10^6	"	1.5×10^5	no growth
Propionic acid (2%)	2.8×10^6	"	3.1×10^5	no growth
Formic acid (1.4%) + paraformaldehyde (0.1%)	2.8×10^5	"	1.8×10^5	no growth
H_2SO_4 (0.3%)	4.2×10^5	"	3.3×10^4	4.0×10^4
B H A (200 ppm)	2.3×10^6	"	4.8×10^5	4.5×10^5
Limestone on top of the grain	3.0×10^5	"	2.2×10^5	6.0×10^4
$CaSO_4$ on top of the grain	1.8×10^5	"	no growth	no growth
Dry molasses on top of the grain	4.0×10^6	"	4.2×10^6	$>300 \times 10^{-4}$
Liquid molasses on top of the grain	3.7×10^6	"	$>300 \times 10^{-4}$	$>300 \times 10^{-4}$
Liquid molasses (7%)	3.2×10^5	"	6.9×10^4	7.4×10^4
Ground corn on top of the grain	3.7×10^5	"	2.8×10^5	4.3×10^5
Ground corn (10%)	7.6×10^5	"	4.2×10^5	6.6×10^5
Lactic acid culture	5.8×10^5	"	9.0×10^4	1.7×10^5

Table 37 (cont'd.)

Treatment	Total count	Coliforms	Lactobacilli	Yeasts and molds
Lactic culture + molasses (7%)	5.3×10^5	no growth	1.0×10^4	4.5×10^4
Lactic culture + ground corn (10%)	3.8×10^5	"	no growth	no growth
Sealed with a plastic bag full of water on the top	5.7×10^5	"	1.1×10^4	8.2×10^5

Lactobacilli population was variable among treatments. The counts ranged from no growth in the control and in the treatment with calcium sulfate on top to a maximum of 4.2×10^6 in the treatment with dried molasses on top of the grain. These values reflect the number of live organisms growing on day 60 and provide no information on microbial population during the fermentation interval up to day 60. Numbers may have been more or less previous to day 60. Lactic acid concentrations of the silage (Table 35) were not proportional to the lactobacilli count at the end of the experiment. Treatments to which a lactic starter was added, did not show an increased lactobacilli count at the end of the experiment. Propionic acid treated grains had a low lactic acid concentration yet had relatively high numbers of lactobacilli. The original number of microorganisms in a silage has been postulated to be inversely proportional to their activity and rate of growth (Weise, 1969).

Yeasts and molds determinations also gave variable numbers for the different treatments. The range was from no growth in the treatments with propionic acid, formic acid plus paraformaldehyde, calcium sulfate on top and lactic acid culture plus 7% molasses, up to a maximum of 6.6×10^5 in the treatment with 10% ground corn. Ethanol concentration in the silage (Table 31) suggests yeast activity during the storage period. Nevertheless, ethanol concentration was more related to the kind of substrate and to the presence or absence of preservatives, than to the final count of yeasts and molds.

Presence of ethanol, acetic and lactic acid indicates the activity of a mixed microbial population, but alcoholic and lactic fermentation predominated in this experiment.

Butyric acid and ammonia were almost absent in the silage, suggesting that the activity of clostridia and proteolitic bacteria was low in the portion of the silage that was not spoiled.

Correlations among measured constituents in Trial 2. Correlation coefficients among the results from trial 2 are presented in Table 39. These coefficients were calculated from averages of values from samples of two barrels on day 32 and one barrel on day 60 (Table 38).

Recovery of good material was negatively correlated with temperature ($r = -.76$). Spoilage caused by aerobic fermentation may have increased the temperature of the grains. Other correlations were not significantly different from zero between spoilage and other measurements. Correlation coefficients between average temperatures and other parameters measured were not statistically significant. Thomas (1976) and Goering et al. (1973) reported high correlations ($P<.01$) between temperatures of stored forage and its acid detergent fiber and acid detergent insoluble nitrogen content. Temperatures in forages ranged from 91 to 108°F, while in the present experiment average temperatures during storage were lower. The range was from 71.7°F in the treatment sealed with a plastic bag full of water to 79.0°F in the treatment to which a lactic culture plus 7% molasses was added. This

Table 38. Average recovery, pH, temperature and composition of wet brewers' grains ensiled for 32 and 60 days. Trial 2[a]

Treatment	% recovery	pH	F° temperature	% DM	% protein	% ADF	% ADN	% ethanol	% acetic	% propionic	% butyric	% lactic	ammoniacal nitrogen
Control	75.2	3.64	77.8	20.3	33.6	25.2	1.02	1.10	0.08	0.02	0.02	2.51	00
Propionic acid (1%)	77.0	3.79	76.8	19.6	35.0	24.4	0.89	0.54	0.24	1.56	0.02	1.74	00
Propionic acid (2%)	97.8	3.99	72.6	21.2	32.6	25.3	0.86	0.23	0.12	3.00	0.02	0.82	00
Formic acid (1.4%) plus paraformaldehyde (0.1%)	96.4	3.28	72.5	23.0	32.0	26.0	1.22	0.02	0.13	0.04	0.00	0.29	01
Sulfuric acid (0.3%)	72.2	2.92	75.2	22.4	31.0	23.0	0.88	0.07	0.10	0.02	0.00	0.26	05
B H A (200 ppm)	68.6	3.59	78.3	21.2	33.9	24.7	0.89	1.43	0.14	0.03	0.00	2.30	01
Limestone on top of the grain	81.2	3.61	72.5	21.0	34.7	24.0	0.90	1.36	0.09	0.02	0.00	1.88	01
$CaSO_4$ on top of the grain	84.0	3.73	73.1	21.8	34.8	25.0	0.88	1.40	0.10	0.02	0.00	1.94	02
Dried molasses on top of the grain	72.4	3.57	75.2	22.2	31.6	24.6	0.84	0.92	0.08	0.02	0.00	2.78	03
Liquid molasses on top of the grain	83.6	3.68	74.3	22.2	34.8	24.6	0.95	0.55	0.08	0.02	0.00	2.36	00
Liquid molasses (7%)	75.0	3.77	74.2	24.4	29.6	20.4	0.76	1.66	0.33	0.04	0.02	2.77	24

Table 38 (cont'd.)

Treatment	% recovery	pH	F° temperature	% DM	% protein	% ADF	% ADN	% ethanol	% acetic	% propionic	% butyric	% lactic	ammoniacal nitrogen
Ground corn on top of the grain	76.6	3.60	74.7	21.9	33.4	25.0	0.82	0.34	0.88	0.02	0.00	2.64	16
Ground corn (7%)	76.1	3.62	78.9	27.1	26.2	19.1	0.62	1.12	0.10	0.02	0.00	2.14	00
Lactic acid culture	69.1	3.72	76.2	21.8	35.4	24.8	0.86	1.08	0.26	0.03	0.00	1.60	02
Lactic culture + molasses (7%)	71.2	3.71	79.0	23.4	32.2	22.3	0.76	2.66	0.31	0.02	0.01	2.25	13
Lactic culture + ground corn (10%)	74.6	3.77	77.2	25.4	29.3	20.4	0.76	1.80	0.35	0.04	0.00	2.06	02
Sealed with a plastic bag full of water on the top	98.5	3.65	71.7	21.2	34.8	24.9	0.85	1.34	0.15	0.03	0.01	1.80	00
Average	79.4	3.63	75.3	22.4	32.6	23.7	0.87	1.04	0.16	0.29	0.01	1.89	04
Fresh brewers' grains		5.00		25.9	31.4	24.2	0.92	0.01	0.07	0.00	0.00	0.14	00

[a] averages of values determined on samples from two barrels on day 32 and one barrel on day 60

[b] % recovery (wet basis), DM = dry matter, % protein (dry matter basis), ADF = acid detergent fiber (dry matter basis), ADN = acid detergent insoluble nitrogen (dry matter basis), % ethanol (wet basis), % acetic acid (wet basis), % propionic acid (wet basis), % butyric acid (wet basis), % lactic acid (wet basis), ammoniacal nitrogen (mg N/100 g wet grain)

Table 39. Correlation coefficients among measured constituents of ensiled wet brewers' grains. Trial 2[a]

X	Y	r
recovery	pH	0.12
	temperature	-0.76**
	dry matter	-0.17
	protein	0.18
	acid detergent fiber	0.40
	acid detergent nitrogen	0.41
	ethanol	-0.37
	acetic acid	-0.29
	propionic acid	0.42
	butyric acid	0.19
	lactic acid	-0.46
	ammonia nitrogen	-0.30
pH	temperature	0.05
	dry matter	-0.06
	protein	0.15
	acid detergent fiber	-0.06
	acid detergent nitrogen	-0.34
	ethanol	0.40
	acetic acid	0.34
	propionic acid	0.46
	butyric acid	0.46
	lactic acid	0.49*
	ammonia nitrogen	0.03
temperature	dry matter	0.31
	protein	-0.36
	acid detergent fiber	-0.47
	acid detergent nitrogen	-0.41
	ethanol	0.41
	acetic acid	0.31
	propionic acid	-0.19
	butyric acid	0.01
	lactic acid	0.36
	ammonia nitrogen	0.04
dry matter	protein	-0.88**
	acid detergent fiber	-0.84**
	acid detergent nitrogen	-0.53*
	ethanol	0.29
	acetic acid	0.32
	propionic acid	-0.33
	butyric acid	-0.30
	lactic acid	0.10
	ammonia nitrogen	0.28

Table 39 (cont'd.)

X	Y	r
protein	acid detergent fiber	0.82**
	acid detergent nitrogen	0.50*
	ethanol	-0.12
	acetic acid	-0.21
	propionic acid	0.11
	butyric acid	0.06
	lactic acid	-0.02
	ammonia nitrogen	-0.26
acid detergent fiber	acid detergent nitrogen	0.75**
	ethanol	-0.47
	acetic acid	-0.50*
	propionic acid	0.22
	butyric acid	0.01
	lactic acid	-0.25
	ammonia nitrogen	-0.36
acid detergent nitrogen	ethanol	-0.50*
	acetic acid	-0.34
	propionic acid	0.01
	butyric acid	-0.01
	lactic acid	-0.45
	ammonia nitrogen	-0.33
ethanol	acetic acid	0.58*
	propionic acid	-0.36
	butyric acid	0.07
	lactic acid	0.55*
	ammonia nitrogen	0.28
acetic acid	propionic acid	0.00
	butyric acid	0.29
	lactic acid	0.12
	ammonia nitrogen	0.42
propionic acid	butyric acid	0.58*
	lactic acid	-0.35
	ammonia nitrogen	-0.21
butyric acid	lactic acid	0.07
	ammonia nitrogen	0.21
lactic acid	ammonia nitrogen	0.36

a correlation coefficients determined on values from Table 38.
* significantly different from 0 (P<.05)
** significantly different from 0 (P<.01)
critical values: α .05 = .48, α .01 = .61

could explain why in the present experiment these relations were not observed.

Values for pH were low for all treatments. Addition of sulfuric acid lowered the pH of the silage to a value of 2.92, but it did not prevent spoilage on the upper part of the barrels in contact with air. Spoilage of this treatment was similar to control. Lactic acid was positively correlated with pH. Addition of acids, particularly formic and sulfuric, lowered the pH of the mass to values around 3.0. Grains from these treatments also had the lowest levels of lactic acid. In other treatments, higher pH values were favorable for the formation of lactic acid.

Silage dry matter was negatively correlated to protein ($r = -.88$), acid detergent fiber (ADF) ($r = -.84$) and acid detergent insoluble nitrogen (ADN) ($r = -.53$), while positive correlations were found between protein and ADF ($r = .82$), protein and ADN ($r = .50$) and ADF and ADN ($r = .75$). Relatively high dry matter values in the grains mixed with molasses and ground corn corresponded to low protein, ADF and ADN contents. Thus, diluting the grain with high dry matter material with low protein, ADF and ADN contents changed these values considerably and may have been partially responsible for the high correlations obtained.

Acid detergent fiber was negatively correlated with acetic acid ($r = -.50$). Addition of molasses or corn to the grains lowered acid detergent fiber values and probably

served as a substrate for the formation of acetic acid. In fact, the highest acetic acid concentrations are found in these treatments.

Correlation coefficient between acid detergent insoluble nitrogen and ethanol was negative ($r = -.50$). Addition of carbohydrate sources (molasses or corn) lowered ADN contents and served as a substrate for the formation of ethanol.

High ethanol values were related to high acetic acid concentrations ($r = .58$) and high lactic acid contents ($r = .55$). Acid treatment of the grain inhibited the formation of these three compounds while the addition of carbohydrate sources increased the content of the same in the silage.

Levels of propionic and butyric acid were low in the silage and positively correlated ($r = .58$).

Summary of Trial 2. In this experiment, 300 lb of wet brewers' grains were stored in steel barrels lined with polyethylene bags. The effect of several additives or methods of ensiling on characteristics of ensiled grains was tested.

Success in ensiling was obtained with the addition of 2% propionic acid, a mixture of 1.4% formic acid plus 0.1% paraformaldehyde or when the grains were sealed with a plastic bag filled with water on top of the barrel. For these treatments, there was no spoilage.

Recovery of good material after 32 days of ensiling was 83% and 68% after 60 days in the control. Covering the grains with limestone, calcium sulfate or liquid molasses improved the recovery of good silage over the control, but

still 17% of the grain was spoiled by day 60. Dried molasses or ground corn layered on top of the grain were somewhat less effective than the aforementioned treatments in preventing spoilage. Addition of and mixing with molasses, ground corn or a lactic acid culture, individually or in combination did not increase the proportion of material recovered as good silage after the ensiling process. Spoilage in the grains treated with sulfuric acid was similar to that of the control.

Fresh brewers' grains had a pH of 5.3. In general for all treatments the pH decreased to a value below 4.0 at day 5 and remained constant until the end of the experiment on day 32 or day 60. No change was observed in dry matter, protein, acid detergent fiber and acid detergent insoluble nitrogen when comparing fresh to ensiled grains. The two main products of fermentation were ethanol and lactic acid. Concentration of acetic acid in the silage was low while propionic and butyric acids were barely detectable. Ethanol and lactic acid formation were inhibited by the presence of acids. Addition of carbohydrate sources increased the concentration of ethanol and lactic acid in the silage. Silage from the treatments with a lactic acid culture had similar composition to the grains having no additive. Ammonia was not formed during the ensiling process in most of the treatments. This, and the fact that protein content did not change with time indicates that there was not protein change or degradation. Temperature of the ensiled grains was positively correlated with spoilage.

Differences in the chemical components of the silage considered good were not proportional to the magnitude of spoilage. Low levels of ethanol and lactic acid were found in good preserved grains when propionic or formic acid were added. Grain to which carbohydrate sources were added had high levels of ethanol and lactic acid but still had extensive spoilage. Sulfuric acid lowered the pH and inhibited the formation of organic acids and ethanol, but did not prevent spoilage in the upper part of the silo in contact with air.

Good conservation of the grains was accomplished by maintaining anaerobic conditions during storage or to the presence of preservatives such as propionic acid, formic acid and paraformaldehyde.

Comparison of Trial 1 with Trial 2

Two storage trials with wet brewers' grains were performed during warm weather. Different methods of ensiling and several additives were tested.

In the first trial 30 lbs of grains were stored in plastic buckets. In a second experiment 300 lbs of grains were stored in steel barrels.

Recovery. After 32 days of ensiling an average loss (spoiled material) of 26% was observed in the first experiment, but only 17% in the second experiment. After 76 days of ensiling, grains in plastic buckets had a loss of 35%, but in the second experiment spoiled material in barrels was only 24% after 60 days of ensiling.

Some preservatives used in the first experiment only slightly reduced spoilage but were essentially ineffective. Complete recovery was achieved in the second experiment when grains kept in barrels were mixed with 2% propionic acid or 1.4% formic acid plus 0.1% paraformaldehyde. When grains were kept under anaerobic conditions in both experiments complete preservation was also obtained.

In both experiments, increases in temperature were related to the amount of spoilage. In Trial 1 grains that had more ethanol had less spoilage, but this did not occur in Trial 2. Schoch (1956) established a positive correlation between dry matter loss and butyric acid concentration in wet brewers' grains silage. This relation was not found in our experiments. There were no dry matter losses when the grains were kept under anaerobic conditions in Trial 1, but butyric acid concentration were similar to other treatments that had considerable losses.

Fermentation pattern. Products of fermentation in the silage considered good varied for the two experiments. Storage in buckets resulted in relatively high concentrations of acetic propionic and butyric acid. Acetic acid content in the grain stored in barrels was low, and propionic and butyric acid were almost absent. There was considerable formation of ethanol and lactic acid in barrel silages while in bucket silages these two compounds were in lower concentrations.

Silage from the grain stored in buckets experienced increases in nitrogen, acid detergent fiber and acid detergent

insoluble nitrogen, and developed large quantities of ammonia. Such changes were not observed in the second experiment, and ammonia when present was in low concentration indicating little proteolysis.

Addition of 10% yeast to the grains stored in buckets resulted in less spoilage, lower pH values and temperatures during the ensiling period, less acid detergent fiber and acid detergent insoluble nitrogen, higher levels of ethanol and lactic acid and lower concentrations of acetic, propionic and butyric acid than grains without yeast. Even so, spoilage losses for the same storage periods were higher than in grain stored in barrels.

Butyric acid, lactic acid and ammonia concentration have been taken as a measure of wet brewers' grains silage quality (Allen et al., 1975). If concentrations of these compounds are taken as criteria of silage quality, then grains with or without yeast kept in buckets were inferior to the grains stored in barrels.

Type of additive. Mineral and organic acids were used as additives in these experiments. Mineral acids (sulfuric acid) lowered the pH and inhibited the formation of volatile fatty acids, ethanol and lactic acid in grains kept in buckets and barrels but did not prevent spoilage. Krinstad and Ulvesli (1951) and Schoch (1957) observed a decrease in dry matter losses when mineral acids were added to brewers' grains and ensiled. This difference is probably due to the type of silo used. Krinstad and Schoch used concrete silos

for their experiments where more anaerobic conditions were maintained than in our experiment.

Propionic acid at a level of 2% and 1.4% formic acid plus 0.1% paraformaldehyde prevented spoilage of grain kept in barrels, but were essentially ineffective at lower levels in grains kept in buckets. Formic acid mixed with paraformaldehyde was more effective in reducing spoilage than formic acid or paraformaldehyde alone in the first experiment. Allen et al. (1975) reported decreased spoilage when formic or propionic acid, or a combination of both were added at a level of 0.40% to wet brewers' grains stored in uncovered piles.

Ammonium isobutyrate increased recovery of haylage ensiled in barrel silos (Thomas, 1976). In our experiments with wet brewers' grains, addition of ammonium propionate or ammonium isobutyrate at levels of 0.5% did not have a beneficial effect on recovery. Increasing the concentrations of these preservatives above 0.5% might produce more desirable results.

Bases such as ammonia or potassium carbonate did not reduce spoilage when added to the grains. Potassium carbonate was added alone or mixed with propionic acid to displace air by means of formation of carbon dioxide. Nevertheless, this did not reduce spoilage of the grains stored in buckets.

The use of carbohydrate sources did not improve preservation in any of the trials. Similar results were reported by Allen et al. (1975 a and b) for grains stored in

test tubes or in uncovered piles. However, Schoch (1957) found that adding 10 to 15% dried apple residue reduced dry matter losses of brewers' grains stored in silos. Brewers' grains mixed with corn have been successfully stored in air-tight silos (Anonymus, 1976). The difference is probably due to the degree of anaerobiosis reached in the silos and amount of material added. In our experiments grains mixed with carbohydrates were kept with the surface exposed to air and that was where spoilage occurred.

Some increases in recovery was observed when a lactic culture was added to brewers' grains stored in buckets. However, addition of a lactic acid culture to grains stored in barrels did not have any beneficial effect. Furthermore, lactic acid concentrations in the grains to which a lactic acid culture was added were not higher than in other treatments. Weise (1969) postulated that activity and rate of growth of microorganisms was inversely proportional to their original number in the silage.

Type of silo. Differences in recovery and fermentation pattern in grains stored in buckets or in barrels are due primarily to the type of model silo used. Surface of exposure to air of grains in buckets was about 850 cm^2 and the weight of the grains was 30 lb while 300 lb of grain stored in barrels had a surface of exposure of about 2400 cm^2. Thus, surface exposed to air per pound of grain was 28 cm^2 in buckets and 8 cm^2 in barrels. Furthermore, during the first experiment, samples were taken periodically from the buckets.

The surface of the grain had to be removed and air was allowed to penetrate in the mass of the grain causing more spoilage.

Test tubes (100 ml) were used to follow fermentation in the second experiment. Allen and Stevenson (1975) compared test tubes and buckets as model silos for brewers' grains and observed that buckets were ineffective in simulating horizontal silo conditions.

In our experiments barrels were more similar in storage conditions to test tubes than to buckets. Nevertheless, some irregular results in pH, ethanol, acetic acid, lactic acid and ammonia were observed in samples of brewers' grains from test tubes. In some samples these values were higher or lower than the concentrations in samples taken from barrels at the end of the experiment. Such differences may be due to the weight of the sample that can be stored in test tubes. A sample of less than 100 g cannot be compacted in the same way as grain stored in barrels. Furthermore, a small sample might not be representative of the large amount of grains stored in a large silo. Air is more easily excluded by the pressure created by the weight of a large mass of grain than by the weight of a small mass.

A small silo, 10 ton capacity, was filled with wet brewers' grains at the same time when the storage trial in barrels was initiated. Grain was covered with a polyethylene sheet over which a layer of corn silage about 20 cm thick was placed. After 60 days of ensiling some spoilage, 2-3 inches, was observed around the edges of the silo but the

rest of the grain appeared well preserved. Liquid was observed draining from the bottom of the silo until day 20. Silage at day 60 appeared drier in the upper part of the silo than in the lower part. Even when no chemical analyses were made on this silage good preservation of wet brewers' grain was apparently achieved by storing it in silos, provided there is adequate sealing.

Practical considerations

In practice wet brewers' grains are delivered to the farms by truck from the brewery where they are placed in some type of storage facility. Grains must be utilized within one week in most storage facilities or they deteriorate and spoil.

In order to prevent losses due to spoilage and decreased nutritive properties, good storage is necessary.

From the experiment described above, using small model silos, wet brewers' grains were completely preserved under oxygen free storage conditions. Spoilage and formation of ammonia and butyric acid were reduced in the silage by increasing size of the silo. An extrapolation of these findings would indicate that wet brewers' grains ensiled in a large silo would have even lower concentrations of ammonia and butyric acid.

Good quality silage can be expected by storage of wet brewers' grains in vertical silos if their construction permits the maintenance of anaerobic conditions. Silo strength and ease of automatic unloading may be a problem when wet brewers' grains are stored in large upright silos.

To store wet brewers' grains in bunker or pit silos, the surface area exposed to air should be minimized. This could be achieved by sealing the grains in these silos with polyethylene sheeting.

The use of preservatives is necessary when the grains cannot be stored under anaerobic conditions. In experiments described above, mixing the grain with propionic acid at the level of 2% or with formic acid (1.4%) and paraformaldehyde (0.1%) resulted in complete preservation of wet brewers' grains silage. Care must be taken in handling these organic acids since they are corrosive and can cause burns in the skin. The preservatives could be mixed with the grains at the brewery since most of the farms do not have mixing equipment. Special facilities at the farm should be built in order to store the grains since existing silos would not always be available for ensiling grains.

When grains to which preservatives have not been added are removed from silos to feed animals, they must be utilized in a short period of time, probably not more than two days, since contact with air will cause rapid spoilage. Grains with added preservatives would be expected to last longer.

The reduction in losses and the increases in quality of ensiled and preserved wet brewers' grains would likely compensate for the additional costs of handling and preserving.

Ensilage of wet brewers' grains will solve the problem caused by fluctuation in supply and demand. A feedable product would then be available when needed and prices would tend to stabilize.

CONCLUSIONS

Wet brewers' grains can be stored for long periods of time if anaerobic conditions are maintained in the silo.

When anaerobic conditions cannot be maintained, the use of preservatives is indispensable. Propionic acid at the level of 2% or a mixture of formic acid (1.4%) and paraformaldehyde (0.1%) are recommended.

Addition of a source of carbohydrates, as corn or molasses, does not exert a beneficial effect on the conservation of wet brewers' grains if anaerobic conditions are not maintained.

Principal products of fermentation of wet brewers' grains are ethanol and lactic acid in barrel silos. In smaller silos there is formation of acetic, propionic and butyric acid, and evolution of ammonia.

Size and type of the silo determine the recovery and quality of ensiled brewers' grains. Small silos produce more deterioration of the silage than larger silos.

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LITERATURE CITED

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