An Evaluation of Chemical Methods To Extend the Allowable Storage Time of Wet Distillers' Grains

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Distillers' wet grains are valuable fermentation byproducts that are extremely perishable. Several chemical preservatives were evaluated as alternatives to energy-intensive, high-temperature drying of the wet grains. Sorbic acid, potassium sorbate, propionic acid, and ammonia at levels of 0.25%, 0.5%, and 1.0% were applied to 100-g samples of wet distillers' grains (61–67% moisture content) that were then held in a laboratory accelerated-storage apparatus. At the 1% treatment level, about 0.4 g of the dry matter was lost when the grains were treated with sorbic acid and held for 21 days. Dry matter losses averaged about 0.4 g when the grains were treated at the 1% level with potassium sorbate and ammonia and held for 9 and 7 days, respectively. Propionic acid was ineffective in our tests. Storage of wet distillers' grains in a static carbon dioxide atmosphere held bacterial counts at initial levels for about 8 days and molds and yeasts for about 16 days.

Each 25.4 kg of No. 2 corn fermented for the production of ethanol will yield from 7.3 to 7.9 kg of moisture-free total byproduct (Bauernfeind et al., 1944b), generally consisting of distillers' grains and distillers' solubles. The relative quantity of these two fractions may vary and is influenced by many factors, some of which are the percentage of grain in the mash, analyses of the grain employed, fermentation efficiency, mash cooking temperature, and the type of equipment used in separating the spent grains from the whole stillage (Bauernfeind et al., 1944a). However, the proportion of distillers' grains to distillers' solubles usually exceeds 50% (Bauernfeind et al., 1944a,b) and may range up to 60% (Waller et al., 1980) on a moisture-free basis. These wet grains, often separated from the solubles fraction by screening, come off the screen containing 85–90% water and may be processed through a dewatering press or centrifuge to yield a material containing 60–70% moisture (*U.S. Dep. Energy Publ.*, 1980). Although wet grains are still quite high in moisture, ways to easily or economically extract more water have yet to be devised. Because of its high moisture content, the storage life of whole stillage or fractions thereof is fairly short, depending primarily on temperature. Higher storage temperatures accelerate spoilage (*Farm Week*, 1980).

One measure of deterioration in feed grains is respiratory activity as determined by the production of carbon dioxide and resultant dry matter loss (Steele et al., 1969). Respiration is slowed in the absence of oxygen, with less carbon dioxide, water, heat, and other compounds such as acetic acid and ethanol being produced. However, the direct effects of respiration on grain are loss in dry matter, gain in moisture content, increase in carbon dioxide in interstitial air, and a rise in temperature (Bothast, 1978). Transposing these principles of grain deterioration directly to spent distillers' grains could be misleading, since spent grains are physically and chemically altered from the feedstock during the fermentation process. However, in these studies the respiration process of spent grains did yield empirical increases in interstitial carbon dioxide as measured from flask headspace samples by gas chromatography. If one assumes a concomitant loss in dry matter, we have a common method by which to measure deterioration of this material when a preserving agent is added.

Allen and Stevenson (1975) reported the efficacy of chemical agents when combined with the ensiling process in preserving spent grains. Lilly et al. (1980) focused on the preservation of spent grains, combining the techniques of adding preservative and reducing the water activity of the material. The objective of our study was to evaluate the efficacy of various preservatives as alternatives to energy-intensive, high-temperature drying of wet grains. The goal was to extend the allowable storage time of this highly perishable feedstuff, allowing a livestock producer more flexibility in using this material in his feeding program.

MATERIALS AND METHODS

The spent grains used were obtained from a commercial ethanol plant designed to produce 800–1000 gal of 95% ethanol/week. In this plant, the corn was ground to pass through a 3-mm mesh screen. After the mash was cooked, converted, and fermented, the beer, containing the fermentation solids, was pumped into the beer column and stripped of ethanol. The hot stillage from the beer column was then centrifuged to separate the spent grains and the liquid-soluble fractions. The moisture content of spent grains recovered in this procedure ranged from 61 to 67% (wet basis). These grains were placed in a polyethylene bag and stored at 1 °C until used.

Experiments were conducted in a laboratory acclerated-storage environment apparatus (Figure 1). Eight 1-L Erlenmeyer flasks were equipped with stainless aeration tubing as illustrated. The aeration inlet port to each flask was connected to an air manifold through 500-mL water-filled gas scrubbing bottles, which supplied humidified air to each of the 100-g samples of wet grains at a constant rate of 10 cm^3/min—equivalent to about 1.0 $m^3\ min^{-1}/ton^{-1}$ aeration rate. The aeration line to each flask was also equipped with an in-line Millipore filter (0.22 μm) to prevent contaminants from entering the flask from the air source. The flasks were held in a constant temperature bath at 30 °C. Carbon dioxide (volume percent) was monitored in the headspace of each flask at regular intervals by using gas chromatography (Ramstack et al., 1979).

Rate of carbon dioxide production was calculated for each headspace sample by multiplying the volume percent value by the air flow rate (10 cm^3/min). A stoichiometric conversion of dry matter (sugar) and oxygen to carbon dioxide and water (i.e., 1.34 g of glucose/L of Co_2) was assumed and the cumulative dry matter lost in each flask was calculated by integrating carbon dioxide production over storage time. Each preservative was evaluated at 0.25, 0.5, and 1.0% weight of active preserving agent/weight of wet grains in duplicate and the results were averaged. Chemical agents tested were propionic acid, potassium

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Table I. Microbial Content of Untreated Spent Grains in Accelerated Storage

storage time, days	pH of grains	microorganisms, mean count/g: total aerobic bacteria count[a]	total anaerobic bacteria count[b]	total *Lactobacillus* count[c]	total coliform count[d]	total yeast and mold count[e]	total osmotolerant count[f]
0 (initial)	4.1	7.7×10^6	1.7×10^6	3.7×10^6	0	6.8×10^5 [g]	8.1×10^5
1	7.3	1.4×10^9	2.8×10^8	3.4×10^8	0	5.1×10^8 [g]	6.5×10^8
3	6.8	2.0×10^8	1.1×10^9	1.5×10^8	0	2.4×10^8 [g]	$>10^8$
6	6.3	8.4×10^9	1.8×10^9	2.8×10^9	0	1.2×10^9 [h]	1.1×10^9
10	6.4	2.1×10^9	1.3×10^9	1.8×10^9	0	9.1×10^8 [i]	5.2×10^8

[a] Determined on standard methods agar. [b] Determined on anaerobic agar. [c] Determined on LBS agar. [d] Determined on Violet Red Bile agar. [e] Determined on yeast extract agar (consisting of 0.4% yeast extract, 1% malt extract, 0.4% dextrose, and 1.5% agar). [f] Determined on malt salt agar (consisting of 2% malt extract, 7.5% NaCl, and 2% agar). [g] Almost entirely yeasts. [h] Predominantly yeasts with some molds such as *Fusarium* sp. [i] Predominantly molds such as *Aspergillus flavus* with some yeasts.

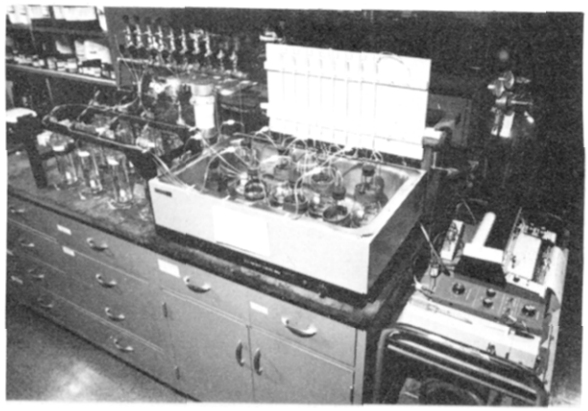

Figure 1. Laboratory accelerated-storage environment apparatus.

sorbate, sorbic acid, diethyl pyrocarbonate, and ammonia (added either as ACS reagent grade ammonium hydroxide or a controlled-release ammonia solution). The controlled-release ammonia solution was donated by Union Oil Co. of California, Brea, CA 92621. The composition of the controlled-release ammonia solution is as follows: 10% ammonia, 40% urea, and 500 ppm of urease enzyme yielding a total ammonia concentration of 30% in the solution. Carbon dioxide was also tested in experiments in which we modified the storage atmosphere in the flasks by substituting CO_2 gas into the aeration/humidification lines in place of air.

Before beginning tests, we conducted a microbiological survey to determine the types and counts of microbial organisms present. One hundred grams of untreated wet grains was placed in each flask in the accelerated-storage apparatus at 30 °C with no preserving agent. Flasks were removed for microbiological analyses after 0, 1, 3, 6, and 10 days of storage. The wet grains were assayed for microflora according to procedures outlined by Bothast et al. (1974).

RESULTS AND DISCUSSION

The microflora present in untreated wet grains used in control experiments and the pH of the grains are summarized in Table I. Counts of microflora made on selective media increased markedly during the first 24 h of accelerated storage and then leveled off. The pH rose rapidly during the first 24 h, during which time no visible spoilage (mycelial growth/color change) could be detected. Spoilage of the grains was generally detected visually by the third day.

Cumulative dry matter losses in the control flasks at 11 days of accelerated storage are shown in Table II. Eleven days in storage was selected as common to all treatments.

Table II. Cumulative Grams of Dry Matter Lost in Preserved Wet Spent Grains during 11 Days of Accelerated Storage/100-g Sample

treatment	preservative level, %: 0	0.25	0.5	1.0
(1) sorbic acid	11.16[a]	8.48	3.17	0.30
(2) potassium sorbate	7.10	9.81	6.91	1.37
(3) ammonia in a controlled-release solution	7.68	6.72	5.93	4.13
(4) ammonia as NH_4OH	11.41	10.22	6.27	1.86
(5) propionic acid	10.89	8.99	10.92	8.11

[a] Each mean is based on two flasks. The LSD (0.05 level) is 3.21.

These losses ranged from 7 to more than 11 g. The variability in these values supports our visual observations that untreated spent grains are biologically heterogeneous and nonuniform in composition. Some flasks would display fairly uniform microbial deterioration, whereas others exhibited pocket or clumps of material more highly susceptible to microbial deterioration. This may be due to "pockets" of yeast and/or bacteria that survived the fermentation/distillation process.

The effects of sorbic acid, potassium sorbate, propionic acid, controlled-released ammonia, and ammonia as ammonium hydroxide on dry matter loss are depicted in Figure 2. Sorbic acid was highly effective in minimizing dry matter loss. At the 1% treatment level, losses were about 0.4 g after 21 days storage. The 1.0% treated material still retained its initial light brown color. A perceptible but faint odor of acetic acid was also present.

Potassium sorbate was moderately effective in minimizing dry matter losses but for a shorter storage time. At the 1% treatment level, losses averaged about 0.4 g after 9 days of storage (Figure 2). These results corroborate the review of Beuchat (1978) in which he stated the antimycotic activity of potassium sorbate is similar to that of sorbic acid, but about 25% more must be added to get the same protection.

Propionic acid was ineffective in extending the storage life of spent grains. Data in Figure 2 show that its use permitted the loss of more dry matter in the treated samples than in the control through about 7 days of storage. These results were expected, since propionates have essentially no inhibitory activity against yeasts (Sauer, 1977). However, because propionic acid has been widely used in the grain industry as a preservative, we wanted to measure its comparative response.

For the controlled-release ammonia solution study, data in Figure 2 show that dry matter losses exceeded 0.5 g after 2 days of storage at all levels of treatment. Ammonia, urea, and controlled-release ammonia solutions have been tested

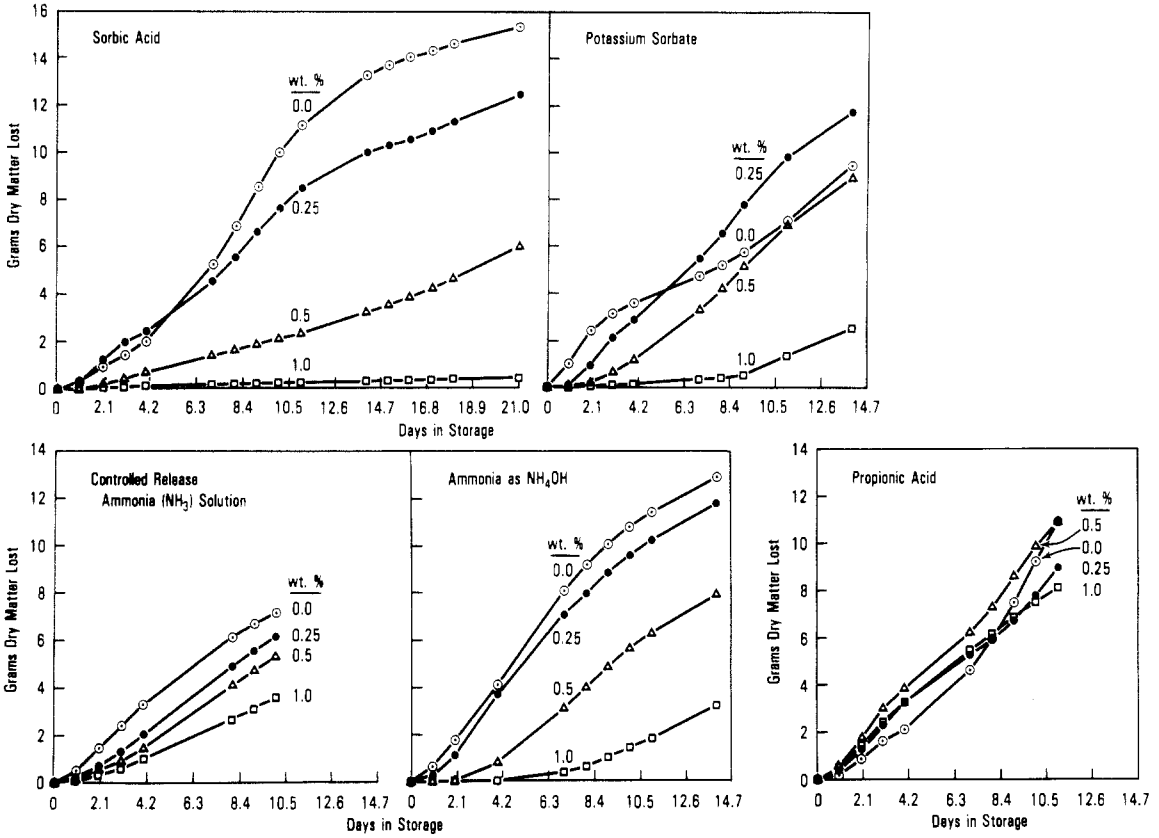

Figure 2. Effects of several chemical preservatives on dry matter losses from 100-g samples of wet distllers' grains in accelerated storage.

as forage preservatives (Knapp et al., 1974; Wilkinson et al., 1978; Ghate et al., 1981; Lechtenberg et al., 1977) and as grain preservatives (Ghate et al., 1980; Bothast et al., 1973, 1975; VanCauwenberge et al., 1980). Ghate et al. (1981) reported that urea did not hydrolyze to release ammonia until a few days after application. VanCauwenberge et al. (1980) reported that a critical amount of ammonia must be present initially for a controlled-release ammonia solution to be effective as a preservative. In our studies, an insufficient quantity of ammonia may have been available initially to provide the necessary control. However, the idea of urea providing controlled-release ammonia for spent grains prservation is worthy of further investigation.

Ammonia, applied as ammonium hydroxide at the 1% level, was a fairly effective short-term preservative. After 1 week of accelerated storage, dry matter losses averaged about 0.4 g (Figure 2). Ammonia has been suggested as an on-farm preservative for wet stillage (*Doane's Agric. Rep.*, 1980). However, it should be kept in mind that ammonia-preserved spent grains are susceptibile to deterioration over an extended period of time.

A three-way statistical analysis was made according to Snedecor and Cochran (1967) of the effect of the level of preservative used on dry matter losses from the spent grains (Table II). At the 1% treatment level, sorbic acid, potassium sorbate, controlled release ammonia solution, and ammonia as ammonium hydroxide showed significant effects. Propionic acid did not display any significant effect at any level tested.

Diethyl pyrocarbonate is reported to have a strong bactericidal effect, decomposing in water to CO_2 and ethanol, and has been suggested as a food preservative (Fedorcsak and Ehrenberg, 1966; Rosen and Fedorcsak, 1966). However, its efficacy is quite short lived. In our tests, its inhibitory effects could not be observed after 1 day in accelerated storage.

Table III. Microbial Content of Spent Grains under Modified CO_2 Atmosphere

treatment	storage time, days	microorganisms, mean count/g: total aerobic bacteria	microorganisms, mean count/g: total yeast and mold
static CO_2	0	7.7×10^6	6.8×10^5
	8	7.6×10^6	7.9×10^6
	16	2.3×10^8	7.9×10^6
	21	3.3×10^8	3.2×10^7
	29	3.0×10^8	3.5×10^5
flow-through CO_2	4	3.0×10^5	2.4×10^6
	15	1.5×10^8	2.5×10^8
	22	2.6×10^8	8.2×10^7
	30	9.9×10^8	5.8×10^7

Christensen and Kaufmann (1974) stated that a carbon dioxide concentration above 14% is detrimental to mold growth, whereas Peterson et al. (1956) reported some growth of molds at an oxygen concentration of about 0.2%. Carbon dioxide has been suggested as an on-farm preservative for wet stillage (*Doane's Agric. Rep.*, 1980). We conducted two studies using carbon dioxide in our accelerated storage apparatus. In the first test (static), carbon dioxide was passed through the aeration/humidification apparatus for about 3 h to purge the system of all other gases. The inlet and exit ports to each flask were then clamped shut and held until sacrificed for microbial analyses, with no further gas exchange. In the second test, carbon dioxide was passed through the apparatus at the same rate as was air in earlier tests—10 cm^3 min^{-1} (100 g of spent grains).$^{-1}$ Both treatments held bacterial counts to initial levels for about 1 week before substantial increases were noted. The static treatment appeared to be somewhat more effective than the flow-through treatment in inhibiting yeasts and molds over the 29-day storage period (Table III).

CONCLUSIONS

(1) Sorbic acid proved to be an effective long term preservative at the 1% treatment level. (2) Ammonia may be an effective short-term preservative. However, technology to effectively apply ammonia to wet spent grains needs to be developed. The Cold-Flo Process now used in adding anhydrous ammonia to silage (Borcherding and Reichenberger, 1977) may have application and needs to be researched. Also, the heat of reaction (chemical) generated when ammonia is added to a high-moisture material such as spent grains, would need to be monitored closely. (3) A comparison of sorbic acid and ammonia shows sorbic acid to be cost prohibitive at the present time. Assuming that 7.6 kg of wet spent grains (65% moisture content) are obtained from each bushel of corn fermented and that ammonia and sorbic acid cost 27¢ and $6.28/kg, respectively, the cost of a 1.0% treatment using these preservatives would be 1.9¢ and 44.7¢ per 25.4 kg of corn fermented. (4) Propionic acid was not effective in preventing dry matter losses at any of the levels tested. (5) Carbon dioxide may find limited application as a shorter term preservative. Some drawbacks to the use of CO_2 as a preservative on spent grains on the farm are (i) its limited bactericidal effect (even when airtight containers are employed) and (ii) the cost of airtight equipment in which to store the spent grains infused with carbon dioxide. (6) Spent grains appear to be nonuniform in terms of microbial deterioration. There may be "pockets" in the mass that are more susceptible to deterioration than the overall quantity of grains. This should be taken into consideration when selecting equipment and methods to be employed in applying preservatives. (7) The use of too little preservative may be more detrimental than applying none at all. In the cases of sorbic acid and potassium sorbate (Figure 2), application of 0.25% of these preservatives resulted in somewhat larger dry matter losses early in storage than was observed in the control sample. This could have been due to selective inhibition of specific microorganisms, which permitted others to proliferate more rapidly. (8) Monitoring the pH of on-farm stored grains may be a relatively simple way of detecting microbial activity prior to visual observation of deterioration. (9) Beuchat (1978) suggested the presence of preservatives in foods may influence the rate of heat inactivation of fungal cells. He observed that as little as 200 ppm potassium sorbate enhanced heat inactivation of ascospores of *Byssochlamys*. This principle may have potential application in the preservation of spent grains. Typical whole stillage comes from the beer column at 80–85 °C and is then centrifuged or passed through a dewatering press to reduce the moisture content; the grains still remain at a relatively high temperature. By utilizing this residual heat, it may be possible to effect a considerable reduction in the amount of preservative needed to control microorganisms, thereby reducing the overall cost of the treatment. Research needs to be directed toward determining optimum levels of preservative needed to treat these fresh, hot spent grains.

Registry No. Sorbic acid, 110-44-1; potassium sorbate, 24634-61-5; NH_3, 7664-41-7.

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