Carbon Dioxide and Aflatoxin Production in High-Moisture Corn Treated with Potassium Sorbate[1]

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ABSTRACT

Potassium sorbate was evaluated as a potential preservative for storage of whole kernel yellow dent corn containing 18, 24, and 30% moisture. Growth of pure cultures of *Aspergillus parasiticus* NRRL 2999 and *Fusarium roseum* (*graminearum*) Mapleton-10 inoculated onto sterile autoclaved corn were monitored by visual observation of mycelial development and carbon dioxide (CO_2) production. Mycotoxin production by the pure cultures was also measured. In general, mycotoxin and CO_2 production were reduced with increasing levels of sorbate. However, samples treated with 0.5% sorbate and inoculated with *A. parasiticus* contained about the same amount of aflatoxin as the control at the end of incubation period. The sorbate was more effective on corn with lower moisture content and in sealed containers, where high concentrations of CO_2 accumulated during incubation. Growth of *F. roseum* was completely inhibited by 0.5% sorbate.

Increasing energy costs have greatly increased the cost of drying corn, and high-moisture grain has been reported to have better feed efficiency than dry grain as livestock feed (Lynch et al 1975). For these reasons it is often necessary or desirable to store high-moisture grain. Temporary storage of high-moisture corn may result in mold spoilage, which decreases dry matter of the grain and introduces potential hazards of mycotoxins. Fungal deterioration of grain causes tremendous annual economic losses (Nichols 1983).

Anaerobic storage of high-moisture corn requires expensive storage facilities. Use of chemical preservatives such as propionic and acetic acids has been shown to have adequate efficacy in feeds and is economical (Christensen 1973). However, corrosiveness to metal surfaces causes problems with the routine use of these chemicals. The antimicrobial properties of sorbic acid and sorbates (sodium or potassium salts of sorbic acid) have long been recognized, and these substances are noncorrosive and generally recognized as safe. In addition, sorbates contribute no odor or taste to foods. Potassium sorbate is used most extensively in foods because it is more soluble in water than sorbic acid. Sorbate is effective at low concentrations as a fungistatic agent in cheese products, baked goods, fruit juices, fresh fruits and vegetables, wines, soft drinks, and pickled products (Luck 1970). Sorbic acid and sorbates have been used to extend the safe storage time of high-moisture corn (Danziger et al 1975, Sauer and Burroughs 1974). Potassium sorbate and sorbic acid have been reported to effectively inhibit aflatoxin production by *Aspergillus flavus* and *A. parasiticus* in corn meal and yeast extract sucrose broth (Masimango et al 1979, Bullerman 1983). The inhibitory effects of potassium sorbate on growth and mycotoxin production by *Penicillium patulum* and *A. ochraceus* also have been reported (Bullerman 1984, 1985).

The objective of this study was to determine the potential effectiveness of sorbate as a preservative for temporary storage of corn with 30, 24, and 18% moisture contents.

MATERIALS AND METHODS

Organisms

A. parasiticus NRRL 2999, a stable, high-aflatoxin-producing strain, and *Fusarium roseum* (*graminearum*) Mapleton-10, a mold common to corn in the midwest, were used in this study. Stock cultures were maintained on potato-dextrose agar at 5°C and transferred every six months.

Inoculum

Conidia of two-week-old potato-dextrose agar slant cultures of *A. parasiticus* NRRL 2999 were harvested by brushing the surface of the slants with a sterile inoculating loop and suspending in 10 ml of sterile phosphate buffer solution containing 0.01% Tween 80 (polyoxyethelene sorbitan monooleate, Fisher Scientific Co.). The mycelial fragments remaining in the spore suspension were removed by filtering through four layers of sterile cheese cloth.

The mycelial mat of *F. roseum*, incubated at 25° C for two weeks in potato-dextrose broth, was separated from the broth media by filtering through Whatman No. 4 filter paper and washing three times with 100 ml of sterile buffer solution. The washed mycelial mat was resuspended in buffer solution and blended at low speed for 1 min to obtain a suspension of spores and mycelia.

Colony-forming units (CFU) of the spore and mycelial suspensions of the two organisms were determined using the plate most probable number method described by Tan et al (1983). The suspensions were diluted to contain final concentrations of approximately 8×10^6 CFU per milliliter for *A. parasiticus* and 1×10^6 CFU per milliliter for *F. roseum*.

Substrates

Nonsterile corn. Whole kernel yellow dent corn was harvested at 30% moisture content and stored frozen until use. Moisture content was reduced to 18% in a portion of the corn using a convection oven (Precision, GCA Corp.) at 35° C. Portions of the nonsterilized 18 and 30% moisture corn were distributed in 100-g quantities into sterile mason pint jars. The jars were closed with standard mason lids modified to contain a silicone (GE auto seal) sealed injection port.

Autoclaved corn. Whole kernel yellow dent corn was obtained at 11.5% moisture content. Straw and stalk debris were removed by hand, and broken pieces were separated from sound kernels with a no. 3.5 sieve (Fisher Scientific Co.) with an opening of 5.644 mm (0.223 inches). Calculated amounts of corn and water to yield a total weight of 100 g at 18, 24, and 30% moisture content were sealed in mason pint jars equipped with standard lids modified as described above. The jars were then autoclaved at 120° C for 15 min and allowed to stand for three days to permit full equilibration of moisture and corn before inoculation with *A. parasiticus* or *F. roseum*.

Inoculation Technique

One milliliter of inoculum suspension of either organism was injected into the jars through the silicone-sealed injection port. A tumbler (Norton, Akron, OH) was used to achieve uniform distribution of inoculum while injecting.

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Sorbate Treatments

A 40% sorbate stock solution was made by dissolving 53.6 g potassium sorbate (Monsanto Co., St. Louis, MO) in water and adjusting the volume to 100 ml. The working solutions for 0.25, 0.5, 1.0, and 1.5% sorbate treatment of corn were prepared by mixing stock solution and water at 5:27, 5:11, 10:6, and 15:1 ratios, respectively. Four milliliters of the appropriate working solution was injected into the jar and distributed by tumbling. For studies involving toxin production, a sterilized filter paper was used to replace the metal jar lid, permitting air diffusion. All samples were incubated at room temperature (25 ± 2° C) and held for 21 days. The study was conducted in duplicate with two replicates.

CO_2 Analysis

Head space gas samples of the incubation containers were analyzed for CO_2 content as a measurement of mold growth (Dixon and Hamilton 1981). A model 8700 Carle basic gas chromatograph (Carle Instruments, Inc., Anaheim, CA) equipped with a molecular sieve column (6 ft × 1/8 in., 42/60 mesh), a Poropak N/Q column (8 ft × 1/8 in., 50/80 mesh), and a thermal conductivity detector were used for CO_2 measurement. The gas chromatograph was calibrated against a standard gas mixture containing 10% CO_2 and 12% O_2 (calibration gas standard, Fisher Scientific Co.) using helium as the carrier gas at a flow rate of 40 ml/min, detector at low position, and column temperature at 120° C. A 0.5-ml sample of gas was withdrawn from each jar and injected into the gas chromatograph using a 1-cm^3 disposable tuberculin syringe. The CO_2 content was calculated from peak areas of standard and samples and expressed as percent by volume.

Mycotoxin Analyses

Samples were taken for toxin analyses on days 12 and 21. Extraction for aflatoxin was done according to the AOAC method 26.029 (AOAC 1980). A silica gel cartridge (Sep-Pak, Waters Associates, Inc., Millford, MA) was used to further purify the extract. Purified extracts were concentrated by flash evaporation under nitrogen and were derivatized with trifluoroacetic acid to convert aflatoxin B1 and G1 into the more fluorescent B2a and G2a forms before analysis by high-performance liquid chromatography (detection limit 3 ppb) (Beebe 1978). The system used was a Varian 5000 liquid chromatograph equipped with C18 column (Waters Associates, Inc.) and a fluorescence detector (Varian Fluorichrom). Zearalenone was analyzed using thin-layer chromatography (detection limit 100–200 ppb) (AOAC 1980).

Statistical Analysis

The studies on aflatoxin production were statistically analyzed by analysis of variance. The significance level was chosen at $P = 0.05$. Data were transformed (natural log) before the statistical analysis.

RESULTS AND DISCUSSION

Carbon Dioxide Production

Respiratory activities of both unautoclaved corn and spoilage microorganisms produce CO_2, which may be an indication of deterioration of shelled corn (Steele et al 1969). When different levels of sorbate were applied to unautoclaved corn, it was found that 1% sorbate completely inhibited mold development for 21 days in corn containing 30% moisture. With corn containing 18% moisture, 0.25% sorbate inhibited mold growth. CO_2 production was reduced by increasing levels of sorbate treatments (data not shown).

Autoclaved corn samples at 30% moisture inoculated with *A. parasiticus* produced sharp increases in the CO_2 content of the head space gas when no sorbate was present (Fig. 1A). A high CO_2 content then inhibited further development of mold mycelia. No sporulation was observed. Head space CO_2 content was reduced with increasing sorbate levels. In addition, the initiation of detectable CO_2 production was delayed with increasing sorbate levels. This was probably caused by the inhibitory effect of sorbate on mold spore germination. After the initiation of CO_2 production, CO_2 content increased much more slowly in samples containing sorbate, indicating that mycelial development was also affected by the presence of sorbate.

Visible mold growth in sorbate-containing samples began with only a few visible colonies. Mycelia then extended from those colonies to eventually cover the entire surface in samples containing 0.5% sorbate. However, with 1.0 and 1.5% sorbate the colonies did not spread or increase in size even after extended incubation.

Corn samples with 24% moisture produced less CO_2 than those with 30% moisture and also had a longer lag period before CO_2 production was observed (Fig. 1B). Rapid increases in head space CO_2 were observed in samples with no sorbate, but maximum concentrations of CO_2 at 24% moisture were lower than at 30% moisture. A rapid increase of CO_2 concentration in samples containing 0.5% sorbate was detected when mold growth became visible. A slight increase in CO_2 production was observed in samples containing 1.0% sorbate, but no visible mold growth was detected.

With corn containing 18% moisture, no significant CO_2 production or visible mold growth was detected in sorbate-treated

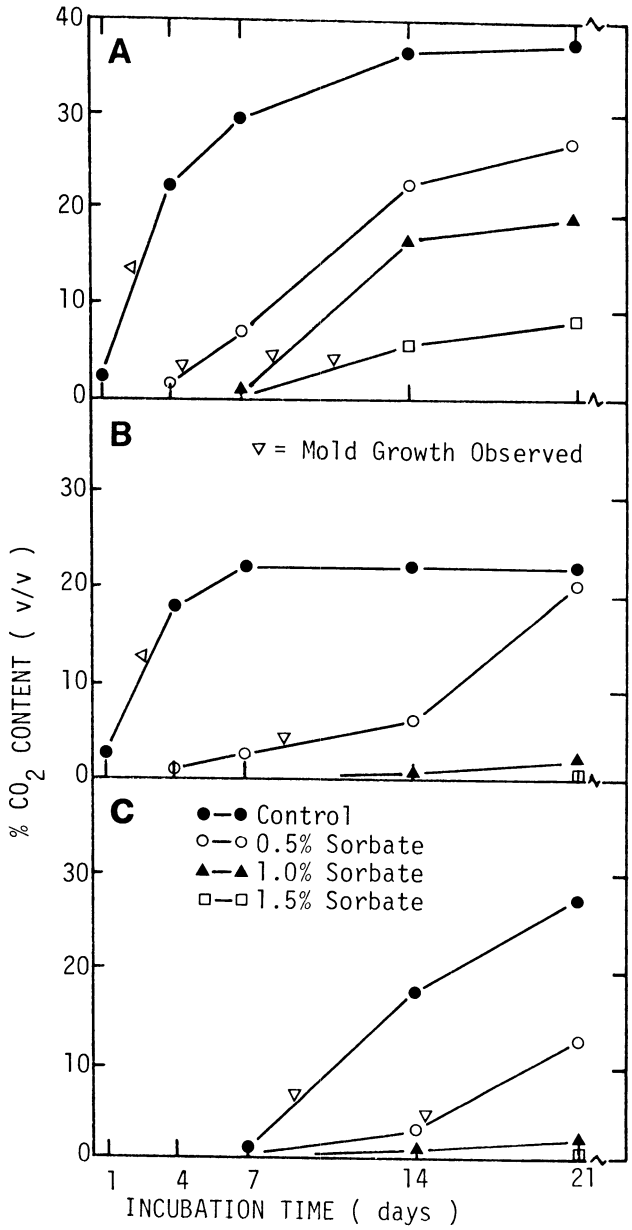

Fig. 1. CO_2 produced by *Aspergillus parasiticus* NRRL 2999 in autoclaved corn treated with sorbate. **A** = 30%, **B** = 24%, and **C** = 18% moisture content.

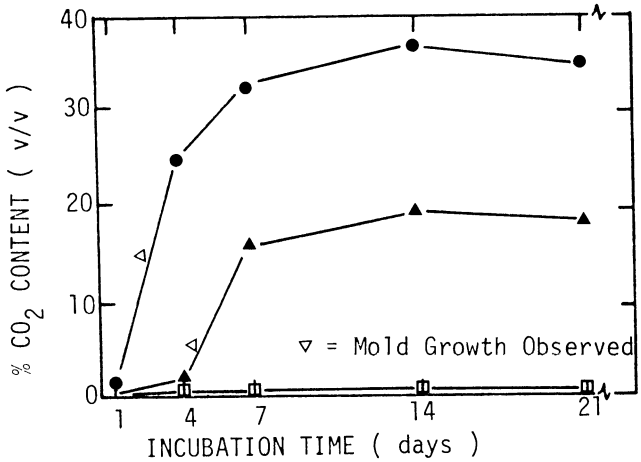

Fig. 2. CO_2 production by *Fusarium roseum* in autoclaved corn without sorbate at 18% (□——□), 24% (▲——▲), and 30% (●——●) moisture content.

samples (Fig. 1C). The CO_2 production in samples with no sorbate treatment at this moisture level was also much slower than at higher moisture levels. Sorbate was more effective in reducing CO_2 production by *A. parasiticus* on corn with lower moisture contents.

F. roseum was more sensitive to sorbate than *A. parasiticus*. Significant CO_2 production was not detected, and no visible growth was observed in any of the samples treated with sorbate (data not shown). With the controls (no sorbate), a distinct lag period for CO_2 production was observed in samples containing 24% moisture (Fig. 2). No CO_2 production or visible growth of *F. roseum* was observed in control samples with 18% moisture. *F. roseum* has a higher water activity requirement than *A. parasiticus* (Brown 1976). Thus, germination and growth were probably stressed or prevented at the lower moisture levels.

Aflatoxin Production

Large amounts of total aflatoxins were produced by *A. parasiticus* on corn containing 30% moisture and no sorbate (Table I). Also, the differences in aflatoxin content in samples treated with 0.5% sorbate and the untreated samples were not significant at the end of incubation period ($P > 0.05$). Although it is impossible to quantitatively measure mycelial mass produced in a solid substrate, it was observed that there appeared to be much less mycelial mass produced at the end of the incubation period in corn containing 0.5% sorbate than in corn without sorbate, even though aflatoxin levels were similar. Stimulation of aflatoxin production by sublethal levels of sorbate has been reported (Bauer et al 1983, Tsai et al 1984). Uneven distribution of sorbate might also allow localized accumulation of high amounts of aflatoxin. Samples containing 1.0% sorbate had greatly reduced aflatoxin contents (less than 1.0% of the aflatoxin produced in the absence of sorbate). Low amounts of aflatoxin, but no visible growth, were found in most samples containing 1.5% sorbate inoculated with *A. parasiticus*. This may have resulted from very limited metabolic activity of the mold or possible carry-over from the inoculum.

In corn with 24% moisture, aflatoxin production was also reduced with increasing sorbate levels in all samples (Table I). The 0.5% sorbate treatment reduced aflatoxin production at day 21 over the control, but the magnitude of the reduction was not statistically significant ($P > 0.05$). At the 18% moisture level, no visible growth of *A. parasiticus* was detected throughout the entire incubation period, but low levels of aflatoxin were detected in all corn samples (Table I). In addition to delaying mycelial growth and aflatoxin production, sorbate also affected the sporulation of *A. parasiticus*. Sporulation was delayed or prevented, and the color of conidia, if any were produced, was yellow to yellowish brown instead of the normal green to dark green color in all samples treated with sorbate. No visible growth of *F. roseum* was observed in corn treated with any level of sorbate or corn containing 18% moisture. Similarly, no zearalenone was detected in any of the

TABLE I
Aflatoxin Production (g/kg) by *Aspergillus parasiticus* in Autoclaved Corn Containing 18, 24, and 30% Moisture and Treated with Sorbate

Moisture Content (%)	Percent Sorbate							
	0		0.5		1.0		1.5	
	12 days	21 days	12 days	21 days	12 days	21 days	12 days	21 days
18	6	12	4	13	5	5	4	6
24	3,234	12,272	86	7,976	7	1,101	3	49
30	11,690	25,616	477	23,702	24	73	4	4

samples of corn inoculated with *F. roseum* at any of the moisture contents, including the control groups with active growth at 24 and 30% moisture. This strain has been reported to produce large amounts of zearalenone only after prolonged incubation at 12° C in addition to an initial incubation at room temperature (Eugenio et al 1970). The experimental conditions used in this study may not have been conducive to production of zearalenone by this strain.

This study showed that potassium sorbate has potential as a preservative for storage of high-moisture corn. However, the effectiveness varied with the moisture content of the corn, the types of molds present, and the storage conditions. The level of sorbate (1.0%) needed to achieve complete inhibition of all the molds studied and to ensure greatly reduced aflatoxin production was too high to be considered economical. However, it may be possible to modify conditions and improve the effectiveness of sorbate or sorbic acid. Also, with more information, it may be possible to use sorbate in combination with other preservative methods (i.e., other chemicals, irradiation, or controlled atmospheres) to achieve more economic and efficient methods of preserving high-moisture grain.

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