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Fermentability of High-Moisture Corn Treated with Chemical Preservatives

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Chemical preservation of high-moisture corn is one alternative to the conventional method of high-temperature drying and has contributed to increased use of high-moisture corn. The present study investigated the use of chemically preserved corn as feedstock for the production of alcohol by fermentation. Preservatives tested were formaldehyde, ammonia, sulfur dioxide, methylene dipropionate (MBP), acetic acid, and propionic acid. Acetic and propionic acids and ammonia-treated corn samples were converted at all concentrations tested, with alcohol production at 80–90% of maximum theoretical alcohol possible. Sulfur dioxide treated corn yielded more alcohol than the other preservatives tested when SO_2 treatments were kept at low concentrations (0.1–0.5%). MBP- and formaldehyde-treated corn yielded low amounts of alcohol and should be avoided as feedstocks for alcohol production.

Increased fossil-fuel prices have stimulated investigations into more economic alternative procedures for conventional high-temperature drying of freshly harvested, high-moisture corn (24–28% moisture content). Chemical preservation of high-moisture corn is one alternative to the conventional method and has contributed to increased use of high-moisture corn. Volatile fatty acids and their salts have received the most attention as preservatives. Propionic acid and mixtures of propionic and acetic acids are presently marketed and prevent mold growth and spoilage in corn containing up to 30% moisture (Hall et al., 1974). Other preservatives which have been investigated include formaldehyde (Muir and Wallace, 1972), ammonia (Bothast et al., 1973; Nofsinger et al., 1977, 1979), sulfur dioxide (Eckhoff et al., 1980), and methylene dipropionate (MBP) (Bothast et al., 1978; Montgomery et al., 1980).

One potential use for preserved corn is as a feedstock for the production of alcohol by fermentation. This study was undertaken to determine the fermentability of high-moisture corn treated with each of six preservatives (ammonia, sulfur dioxide, MBP, propionic acid, acetic acid, and formaldehyde) at four concentrations (0.1, 0.2, 0.5, or 1.0% w/w) with untreated corn as a control. The four concentrations used are those that might actually be employed to preserve corn "in the field". It should be noted that, even at the same concentration level, the chemicals employed are not equivalent as antimicrobial agents.

MATERIALS AND METHODS

The corn used in this experiment was freshly harvested high-moisture corn (28% moisture level) that was stored at 0 °C until used. Samples (400 g) were placed in 2-L Erlenmeyer flasks and brought to ambient temperature. The various preservatives were then added (Table I) to the corn on a weight of active preserving agent to weight of corn basis. The flasks were sealed and kept at ambient

Table I. Quantity of Preservative Used to Reach the Desired Concentration on 400 Grams of High-Moisture Corn[a]

preservative	concentration of preservative			
	0.1 %	0.2 %	0.5 %	1.0 %
acetic acid	0.4[b]	0.8	2.0	4.0
ACS ammonium hydroxide	1.33	2.66	6.67	13.34
formaldehyde	1.16	2.32	5.80	11.60
MBP	0.4	0.8	2.0	4.0
propionic acid	0.4	0.8	2.0	4.0
sulfur dioxide	0.4	0.8	2.0	4.0

[a] The concentrations were calculated from the "wet" weight of the corn. [b] Values are in grams.

Table II. Protocol Followed for Preserved, High-Moisture Corn Fermentations

step 1: add 162.4 g of treated corn to 560 mL of distilled water in a 1-L Erlenmeyer flask
adjust the pH to 6.2
add 0.32 mL of Taka-therm α-amylase
heat to 90 °C with stirring
maintain at 90 °C for 1 h
step 2: cool by adding 150.4 mL of distilled water
reduce temperature to 60 °C
adjust pH to 4.0
add 1.2 mL of Diazyme L-100
maintain at 60 °C for 2 h
step 3: cool to 32 °C
adjust pH to 5.0
add yeast inoculum, 1% v/v
allow to ferment for 3 days at 32 °C

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Table III. Alcohol Production on Chemically Treated Corn

preservative	preservative concentration on corn				
	0.0%	0.1%	0.2%	0.5%	1.0%
acetic acid		4.6[a] (82.4)[b]	4.9 (87.8)	4.8 (86.0)	5.0 (89.6)
ammonia		4.7 (84.2)	4.7 (84.2)	4.3 (77.1)	4.3 (77.1)
formaldehyde		4.5 (80.7)	0.0	0.0	0.0
MBP		4.2 (75.3)	3.9 (69.9)	3.6 (64.5)	0.0
propionic acid		4.8 (86.0)	4.3 (77.1)	4.6 (82.4)	5.0 (89.6)
sulfur dioxide		5.4 (97.7)	5.5 (98.6)	4.8 (86.0)	0.0
control	4.8 (86.0)				

[a] All values are the average of duplicate fermentations and represent values on a weight of alcohol per weight of sample basis. The LSD at the 0.05 level is 0.63. [b] These values (in percent) represent the ethanol conversion efficiency based on a theoretical maximum of 5.58%.

temperature for 2 weeks. All preserved samples, and a control, were coarse ground by using a Hobart Granulator coffee mill (the material passed through a U.S. Standard No. 4 mesh screen, a 4.76-mm opening). Each sample was mixed thoroughly and fermented according to protocol (Table II). The 90 °C temperature in step 1 was attained in a steam cabinet, with manual stirring at 15-min intervals. The enzyme used was a commercial bacterial α-amylase with an optimum pH range of 5.5–7.0 and an optimum temperature range of 80–95 °C (176–203 °F). A water bath was used, to maintain the 60 °C temperature in step 2. The enzyme used in this step was a fungal glucoamylase with an optimum pH range of 3.8–4.5 and an optimum temperature range of 50–60 °C (122–140 °F). The pH was adjusted by using either a dilute NaOH solution or a dilute HCl solution.

The yeast inoculum was prepared by inoculating 100 mL of yeast malt broth (yeast extract, 0.3%, malt extract, 0.3%, peptone, 0.5%, and dextrose, 1.0%) with a loopful of cells from a stock slant of *Saccharomyces uvarum* NRRL Y-1347. This broth was incubated at 32 °C for 3 days before use in the experiment. After inoculation and during fermentation each test flask was sealed with an Alwood valve containing concentrated sulfuric acid, which prevents alcohol loss while allowing the CO_2 gas evolved to escape. At the end of the 3-day fermentation period, each sample was assayed for ethanol on a Varian 3700 gas chromatograph equipped with a 6-ft Porpak Q column operated at 190 °C and for glucose with a Waters ALC-201 HPLC having a Bio-Rad HPX-42 gel filtration column and a refractive index detector.

RESULTS AND DISCUSSION

Theoretical ethanol yields were calculated by (1) assuming complete stoichiometric conversion of starch to glucose and subsequent fermentation to ethanol and (2) allowing a loss of 5% of the available glucose to yeast cell production (Bothast and Detroy, 1981). The final theoretical ethanol yield possible was 5.6%. This figure was used in Table III to calculate the percent of theoretical obtained from the actual values arrived at from GLC analysis. Replicate fermentations were consistent over all treatments.

Of the preservatives tested, formaldehyde was the most detrimental to the fermentation process. Only at the 0.1% level was any alcohol produced (4.6%, or an 80.7% conversion of available glucose). At the end of the test period glucose was detectable only in fermentations that were completely inhibited, i.e., those substrates treated with sulfur dioxide or MBP at the 1.0% level or with formaldehyde at the 0.2, 0.5, or 1.0% levels. These 1.0% sulfur dioxide and MBP treatments were enough to inhibit cell growth but not to interfere with enzymatic conversion. However, formaldehyde did apparently interfere with conversion because only an average of 7.3% glucose was detected at the end of the test compared to 9.9 and 9.4%, respectively, for 1% sulfur dioxide and 1% MBP.

The alcohol produced from the MBP-treated corn dropped considerably as the MBP concentration increased above 0.5%. Alcohol (4.2%) (weight of alcohol/media) was produced from the 0.1% treatment, 3.9% alcohol was produced from the 0.2% treatment, and only 3.6% alcohol was produced from the 0.5% treated corn. No alcohol was produced from the 1.0% MBP-treated corn.

The acetic acid and propionic acid treated corn were both fairly consistent in the amount of alcohol produced, regardless of the preservative level. The acetic acid treated corn averaged 4.8% alcohol and propionic acid treated corn averaged 4.7% alcohol over the four experimental treatment levels. The 1.0% treatment levels for both actually showed the highest alcohol content (5.0%).

Ammonia-treated corn fermented approximately the same at the 0.1 and 0.2% levels (4.7% alcohol produced for both) but was slightly lower for both the 0.5 and 1.0% levels (4.3% alcohol produced for each). The higher ammonia levels may inhibit the inoculum growth.

Sulfur dioxide treated corn was very efficient as a substrate in alcohol production at both the 0.1 and 0.2% treatment levels, producing 5.4 and 5.5% alcohol, respectively. Previous research has shown that sulfur dioxide breaks down the protein matrix that binds the starch (Cox et al., 1944; Wagoner, 1948), allowing more starch to be available for conversion to glucose by the enzymes. This is a possible explanation for the increased alcohol production at this level. The 0.5% treatment level was slightly less productive (4.8% alcohol) but was still visibly fermenting when removed for sampling. Corn treated with 1.0% sulfur dioxide did not ferment, but the starch was efficiently converted to sugar and yielded a 9.9% (w/v) solution.

In conclusion, sulfur dioxide treated corn yielded more alcohol than the other preservatives tested, as long as the SO_2 treatment was kept at low concentrations (0.1–0.5%). Corn samples treated with acetic and propionic acids and ammonia were converted at all the concentrations tested, with alcohol production at 80–90% of the maximum theoretical alcohol possible. MBP- and formaldehyde-treated corn yielded low amounts of alcohol and should be avoided as feedstocks for alcohol production.

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Effect of Magnesium Fertilization on the Quality of Potatoes: Total Nitrogen, Nonprotein Nitrogen, Protein, Amino Acids, Minerals, and Firmness

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The effect of magnesium fertilization on total nitrogen, nonprotein nitrogen, protein content, and amino acid composition as well as firmness and mineral content of Katahdin potatoes was examined. Magnesium sulfate was applied at rates of 0, 40, and 100 lb/acre. At all levels of fertilization, total nitrogen, protein, and the summation of both free and total amino acids of tubers were increased. Nonprotein nitrogen and mineral content varied with the year of cultivation. Tubers from plants receiving magnesium fertilization were significantly firmer than controls.

Magnesium is an essential nutrient for plant growth and metabolism and is required for the translocation of sugars in potato plants (Lewin and Lewin, 1956). Magnesium sulfate fertilization has been shown to increase anaerobic respiration, decrease oxygen consumption, and increase carbon dioxide evolution (Vermes et al., 1974). The magnesium content of potato tubers was shown to increase following $MgSO_4$ application (Vermes et al., 1974). Although the phosphorus content of tubers was depressed by $MgSO_4$ fertilization, there was no observed effect on the contents of nitrogen, potassium, calcium, or magnesium (Laughlin, 1966); however, levels of fertilization were very high (250 and 500 lb/acre). The addition of magnesium to a nitrogen–phosphorus–potassium fertilizer treatment reduced the potassium and manganese contents and increased the magnesium content of snap bean leaves in two experiments, while leaf nitrogen was also reduced and leaf phosphorus increased during one of the two experiments. The magnesium content of the total plant was increased during both experiments, while total plant potassium and manganese contents were reduced during only one (Polaniyandi and Smith, 1978).

Since previous work from our laboratory has shown that the fertilization with $MgSO_4$ increased yield, reduced discoloration and phenolic content, and increased crude lipid and phospholipid content of potato tubers (Klein et al., 1981), this study was conducted in order to determine the effect of $MgSO_4$ fertilization on the contents of total nitrogen, nonprotein nitrogen, protein, amino acids, minerals, and firmness of Katahdin potato tubers.

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MATERIALS AND METHODS

Katahdin potatoes grown at the Cornell Vegetable Research Farm at Riverhead, Long Island, NY, during the 1978 (year 1), 1979 (year 2), and 1980 (year 3) growing seasons were used in the study. Soil type was Riverhead fine sandy loam. Magnesium sulfate was banded at planting at rates of 0, 40, and 100 lb/acre. The randomized block design contained two replicated plots per treatment. The pounds per acre of available minerals on these plots averaged as follows: magnesium, 70; phosphorus, 43.4; potassium, 219; calcium, 2240; manganese, 12.6; zinc, 2.4. The soil was not deficient in any of these minerals for potato crop (Kelly, 1981). Soil organic matter averaged 2.91%, and soil pH was 6.1. All plots were irrigated in the same manner.

Tubers were harvested 24 weeks after planting and stored at 5 °C for 5 months prior to analysis. Uniform tubers of medium size were sliced longitudinally from bud to stem and then divided into cortex (including the periderm) and pith sections, frozen, lyophilized in a Stokes freeze-dryer, ground in a Wiley mill through a 40-mesh screen, and stored under nitrogen until analyzed. Cortex tissue was used for all determinations because of its high metabolic activity.

Determination of Total Nitrogen Content. The method described in AOAC (1975) was used for total nitrogen determination. Duplicate determinations using 100 mg of freeze-dried powder were made on each treatment.

Determination of Nonprotein Nitrogen Content. Nonprotein nitrogen was determined using a modified version of the method of Desborough and Weiser (1974) as previously described by Klein et al. (1980). Duplicate determinations were made on each treatment.

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