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Slowing the mold growth on stored corn: The effects of vinegar, baker's yeast, and yogurt on corn weight loss

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Summary

There are many methods for preserving grain, most of them chemical. The application of chemical preservatives, such as strong acids or antifungal chemicals, has negative effects on farm storage facilities, animals, and humans. Biologically and environmentally friendly inhibitors can reduce the growth of harmful fungi and molds while also having nutritional benefits. In this study, three substances were used to study mold growth on corn kernels. Vinegar, baker's yeast, and yogurt were applied to corn. Tap water was used as a control. Prior to adding these three substances the corn was steeped to reach a 30% moisture level and stored in Ziploc- plastic bags at room temperature (approximately 25ºC). Bags were weighed once a week and weight losses were recorded. Weight loss after four weeks was highest in the water treatment and lowest in the baker's yeast treatment. Both the yogurt and baker's yeast treatment were associated with more weight loss during the first week than the other two treatments, weight loss being a measure of mold growth. However, the baker's yeast and yogurt treatments were associated with the least amount of weight loss during the last two weeks of incubation. Vinegar was very successful at preventing weight loss during the first week of incubation but the corn lost weight at a rate similar to that of the control during the following three weeks. In conclusion, biological treatments were as successful as chemical treatments in slowing down mold growth in corn after four weeks of incubation.

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Introduction

Today's society is reluctant to introduce large amounts of chemical preservatives into the food chain (1). Biological inhibitors have shown promise for reducing grain spoilage, while also being more sustainable (2). These compounds allow for less dependence on manufactured preservatives.

There are chemical and biological inhibitors of mold growth. Mold growth has important implications for both agriculture and human health. Molds cause great economic losses due to food and feed spoilage. Fungal deterioration causes 5 to 10% of the world's food production to be lost every year (3). Fungal growth not only decreases the amount of food available, but also may produce mycotoxins (4). Mycotoxins are secondary metabolites produced by microfungi that are capable of causing disease and death in humans and other animals (5). The FAO estimates that mycotoxins affect 25% of the world's crops (6). Economic losses due to mycotoxins in animal feed are substantial. It has been estimated that just in the USA mycotoxins cost $932 million per year (7). In Asian countries, aflatoxins, one of the most common types of mycotoxins, are estimated to cost $900 million per year (8).

Farmers have used several methods to combat losses due to microbiological growth, including drying of grain, application of chemical preservatives, and use of biological inhibitors (9). Newly harvested grain contains high amounts of moisture. Artificial drying requires the use of fossil fuels and may crack the grain kernels, potentially facilitating fungal attack (10). Organic acids are an alternative to artificial drying. In this method, compounds such as acetic acid are used to reduce the pH of the grain, which inhibits the development of molds and fungi (9). Major drawbacks to this method are the corrosive nature of the acids on metal structures, such as silos, and its potential effects on workers' safety. The direct corrosive effect of the acids on the skin and the acids' effects on the respiratory system due to their volatility make them dangerous to handle. Another chemical treatment is the use of ammonia gas as an antimicrobial agent (9). The use of ammonia gas is also dangerous to workers and can destroy the nutrient quality of the grains or affect the flavor of the products.

The use of biological inhibitors has a long history in agriculture for the reduction of grain spoilage. These methods leverage certain microbial species' ability to inhibit the growth of competing organisms. The interaction between species of microorganisms that are competing for the same resources is termed the competitive exclusion principle (11). Certain microorganisms used as biological inhibitors may also enhance food quality and add nutritional value to the end product, acting as probiotics (12).

When deciding on the use of a method to reduce the population of harmful microbial species, several characteristics should be considered (9):

1. It should have a very low toxicity to humans and animals.
2. It should not negatively affect the nutrient value of the grain.
3. It should consist of compounds that are metabolized by known pathways, as metabolites of the compounds should not be toxic.

Week	Treatments				Average across treatments
	Control	Baker's Yeast	Vinegar	Yogurt	
1	0.65%	0.88%	0.36%	0.69%	0.65%
2	0.75%	0.56%	0.66%	0.72%	0.67%
3	0.75%	0.36%	0.66%	0.56%	0.58%
4	0.85%	0.36%	0.59%	0.59%	0.59%
Weekly average	0.75%	0.54%	0.56%	0.64%	0.62%
Total losses	2.98%	2.15%	2.26%	2.56%	

Table 1. Weekly weight loss due to mold growth in corn treated with no additives (control), vinegar, baker's yeast, and yogurt.

As grain preservation has major economic and environmental impacts, the objective of this experiment was to investigate whether the competitive exclusion principle in microbiology was a feasible method of corn preservation when compared to chemical mold inhibitors for the preservation of corn grain. The competitive exclusion method was tested using yogurt and baker's yeast, while vinegar (5% acetic acid) was tested as a chemical preservative. To speed up mold growth, corn moisture was increased to 30%.

Results

Weight loss is a good approximation of mold growth because as mold grows it uses the mass of the corn to sustain itself. However, not all corn mass is transferred to mold mass, as part of that mass is transformed to CO_2 due to normal mold respiration (13). The corn in all treatments lost weight during the first week of incubation (0.64% across treatments), suggesting that mold growth was taking place, although mold was not visible until the last two weeks of incubation. All treatments showed visible mold growth after 4 weeks of incubation. Data collection was stopped at that time, as visibly molded corn is not considered fit for consumption by either humans or animals (**Figure 1**). Average weight loss peaked at 0.67% during week 2 of incubation (**Table 1**). Weight loss decreased to 0.58% and 0.59% in weeks 3 and 4, respectively (**Figure 2**). At the end of the fourth week of incubation, the baker's yeast treatment yielded the lowest cumulative weight loss (2.15%), followed by the vinegar (2.26%), the yogurt (2.56%), and the control (2.99%).

Discussion

Baker's yeast slowed mold growth the most, while the water control showed the highest weight loss. Yeast may have competed with the mold and slowed its growth, possibly showing the effects of competitive exclusion. Biological inhibitors worked best during the last two weeks of the experiment, when either the baker's yeast or the bacteria in yogurt had grown and were competing with the wild mold (**Table 1**). Although it is not possible to disentangle corn mass loss from baker's yeast and mold mass gain, the weight losses in the yeast treatments were equal to or numerically greater than in the control treatment during the first week of the experiment, which may indicate extra losses from growth of the added microorganisms. Once the substrate available on the surface of the corn ran out, both baker's yeast and wild yeast may have stopped growing. Wild yeast is not only present on the surface of the kernel, but also in the interior (14) and as there are several species of wild mold, these wild species may use more varied substrates than baker's yeast. The same growth dynamics may also apply to the yogurt treatment. However, in this latter case, weight loss was higher than either the baker's yeast or the vinegar treatments. Vinegar was very successful at slowing mold growth during the first week of the experiment, but was inferior to the baker's yeast

Figure 1: Molded corn after 4 weeks of incubation.

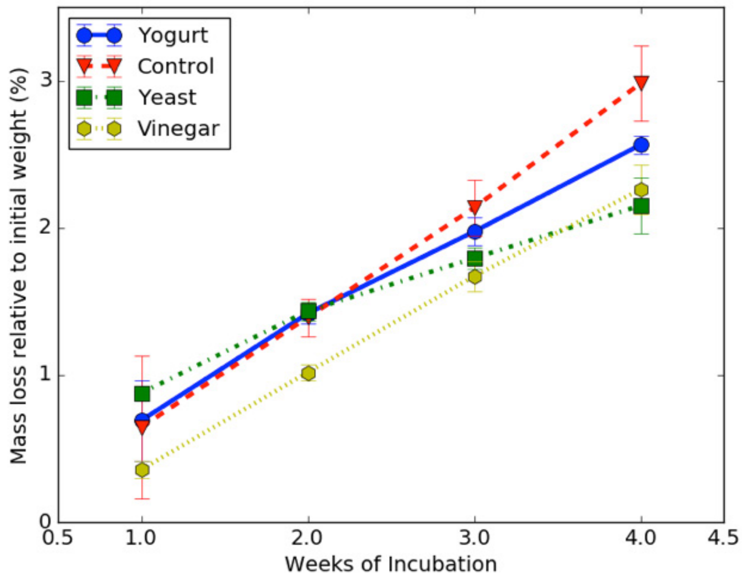

Figure 2. Cumulative weight loss due to mold growth in corn treated with water (control), vinegar, baker's yeast, or yogurt. Vertical bars indicate standard deviation.

and yogurt treatments in later weeks. Vinegar may have lost its anti-mold properties after the first week.

Although this experiment accelerated mold growth by increasing the corn's water content to 30%, corn is usually harvested below 20% moisture (15). Therefore, these results should be taken with caution as the model used may not completely reflect current commercial storage conditions. Future experiments in this area could be used to try to better mimic real-life situations by using corn with lower levels of moisture and longer storage times. Another area of research could be the effects of these biological additives on mold growth inhibition in other grains such as wheat, barley, or rice. Finally, the possible modes of action are currently speculative because no direct measurement of mold growth was done in this experiment. The inhibition could also be indirect, caused by substances produced by the yeast or lactobacilli. Baker's yeast secretes toxins that inhibit other yeasts and fungus (16), and lactobacilli produce organic acids (17) and other organic molecules (18) that also have been shown to inhibit molds. Other modes of action, such as the growth of additional microorganisms that might have inhibited mold growth, are also possible.

Baker's yeast most effectively slowed mold growth after 4 weeks incubation when compared to other treatments. The use of biological microorganisms to avoid mold growth through competitive exclusion should be further studied as a possible substitute for current chemical methods, such as acetic acid.

Methods

Corn of the popcorn variety was used because of its known moisture content (13.5%). Moisture in the corn grain was raised to approximately 30% to accelerate fungal growth. This was done by first steeping 1200 g of dry corn with 120 g of water overnight. Then, approximately 90 g of corn was mixed with 11 g of either pure water or water with dissolved vinegar, yogurt, or baker's yeast. The final moisture content of corn averaged 31% across treatments. Samples averaging 105 g of high-moisture corn were then placed into one-quart Ziploc bags and separated into triplicates by treatment. Although the Ziploc bags were closed, they were gas permeable (19). Gas exchange through the plastic walls allowed for calculation of mass loss. Bags only "puffed" during the first week and were weighed "unpuffed".

Treatments were: water, vinegar, yogurt, and baker's yeast. Vinegar (Heinz vinegar, 5% acetic acid according to the company's label) was used as the source of acetic acid to reach a final concentration of 0.05% of total sample weight. Yogurt (Fage Greek Yogurt: Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, and Lactobacillus casei according to the company's label) was used as the source of lactobacilli to reach a concentration of at least 10^9 CFU/gram of total sample. Finally, baker's yeast (Red Star Active Dry Yeast) was used to reach a concentration of 10^{10} CFU/gram of total sample. Bags were weighed weekly to calculate losses due to microbial growth and kept at room temperature on a laboratory bench, as corn is stored unrefrigerated and room temperature is the most typical temperature to store food items.

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