Journal of Food Protection, Vol. 50, No. 11, Pages 909-914 (November 1987)

Growth and Aflatoxin Production by *Aspergillus parasiticus* NRRL 2999 in the Presence of Acetic or Propionic Acid and at Different Initial pH Values

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(Received for publication February 13, 1987)

ABSTRACT

Experiments were done to determine effects of different concentrations of acetic or propionic acid in a glucose-yeast extract-salts medium with an initial pH value of 4.5 or 5.5 on growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999. Amounts of aflatoxin were measured with reversed-phase high-performance liquid chromatography. The maximum concentration of acetic or propionic acid that permitted growth at an initial pH of 5.5 was 1% after 7 d of incubation and 0.25% after 3 d of incubation, respectively. When the initial pH of the medium was 4.5, the maximum concentration of acetic or propionic acid that permitted growth was 0.25 or 0.1%, respectively. There was no significant difference ($p>0.05$) in amount of mycelial (dry weight) produced by cultures in the presence of 0.0, 0.25, 0.50 or 0.75% acetic acid. Amounts of aflatoxin B_1 and G_1 produced decreased with an increasing concentration of acetic acid. Increasing concentrations of propionic acid caused a decrease in the amount of mycelial dry weight and aflatoxin produced by cultures growing in the medium with an initial pH of 5.5. At an initial pH of 4.5 mycelial growth was slow and at 3 d of incubation amounts of aflatoxin B_1 and G_1 produced were reduced as concentrations of acetic acid increased. This also was true for propionic acid in the medium with an initial pH of 4.5. Cultures with an extended lag phase in the presence of acetic or propionic acid overcame this and then produced large amounts of aflatoxin B_1 and G_1 at 7 and 10 d of incubation.

Aflatoxins are a group of toxic fungal metabolites produced by several species of *Aspergillus*. There are at least 16 naturally occurring aflatoxins, but the four major toxins are B_1, B_2, G_1 and G_2. Aflatoxin B_1 is a potent hepatocarcinogen, mutagen and a teratogen *(6,14)*. Strains of *Aspergillus parasiticus* and *Aspergillus flavus* that produce aflatoxins can do so under diverse environmental conditions *(16)*, on a variety of agricultural and food commodities *(4,15)* and in the presence of certain concentrations of numerous preservatives *(24)*.

Volatile organic acids such as acetic and propionic are commonly used in the food industry as preservatives. Acetic acid is more effective in limiting yeast and bacterial growth than mold growth *(9)*. Levine and Fellers *(20)* showed that *Bacillus, Salmonella* and *Staphylococcus* were inhibited by lower concentrations of acetic acid than were *Saccharomyces* and *Aspergillus*. In the presence of sugar and brine, 0.9% acetic acid prevented growth and toxin production by *Clostridium botulinum* in whole pickles *(18)*. Woolford *(33)* observed that at pH values of 4, 5 and 6 heterolactics were more resistant to acetic acid than homolactics. Furthermore, *Bacillus* and gram-negative bacteria were more sensitive to acetic acid than lactic acid bacteria, yeasts and molds; at pH values below 4, these organisms were equally inhibited. Acetic acid at pH 3.4 was effective against the bread molds, *Aspergillus niger* and *Rhizopus nigrificans*. The amount of acetic acid required to inhibit *Aspergillus fumigatus* decreased as the pH was lowered *(19)*. A 1% concentration of acetic acid at pH 4.5 completely inhibited growth and aflatoxin production by *A. parasiticus*. Concentrations of 0.6 or 0.8% partially inhibited growth and decreased toxin formation by 70 and 90%, respectively *(3)*.

Propionic acid and the propionates are highly effective mold inhibitors but have little or no effect against yeasts, which is why these chemicals are used in the baking industry *(26,28)*. Several studies have dealt with use of propionates to control mold growth in stored grain. Sauer and Burroughs *(27)* reported that propionic acid and sodium propionate were more effective than calcium propionate in preserving corn containing 18% moisture. Furthermore, calcium propionate at 0.5 and 1.0% delayed growth of mold by 1 week, whereas propionic acid and sodium propionate at similar concentrations delayed initiation of mold growth by 17 weeks. Bandelin *(1)* observed that at pH 5.0 from 0.06 to 0.08% propionic acid was required to inhibit several molds, including *A. niger*. According to Vandegraft et al. *(32)* 1% propionic acid effectively prevented growth and toxin production by *A. flavus, A. parasiticus, Aspergillus ochraceus* and *Penicillium viridicatum* in artificially inoculated corn for up to 29 weeks of storage, whereas *A. parasiticus* and *A. ochraceus* grew excessively in untreated corn.

Buchanan and Ayres *(3)* observed that 0.1% propionic acid partially inhibited growth and aflatoxin production by *A. parasiticus,* whereas complete inhibition was achieved by 0.2%. Masimango et al. *(23)* reported that 0.5 and 1.0% of propionic acid inhibited aflatoxin production by 49.2 and 52.3%, respectively, whereas with 1% calcium propionate only 33.1% inhibition was achieved. No inhibition of growth or aflatoxin production by *A. flavus* occurred with 0.1% propionic acid or calcium propionate. Data by Stewart et al. *(30)* show that 0.0003% propionic acid in a liquid medium inhibited germination of *A. parasiticus* conidia but did not affect their viability, whereas 0.0004% inactivated the conidia.

Organic acids exert their effect through undissociated molecules *(2,8,17,20)*. Consequently, the activity of the acids is pH-dependent, because pH determines the degree of dissociation. Hence, effective use of an organic acid depends upon the dissociation constant (pK_a) or the pH at which 50% of the total acid is undissociated. Since the undissociated portion of the molecule is believed to be responsible for the antimicrobial effect, it is advantageous to use and study the acids near these values. Thus, when designing this study, the initial pH of the test substrate was near the pK_a of the acid being evaluated.

This study was done to determine the effect of various concentrations of acetic and propionic acids on growth and aflatoxin production by *A. parasiticus* NRRL 2999 at 28°C in a glucose-yeast extract-salts medium at pH 4.5 or 5.5.

MATERIALS AND METHODS

A preliminary experiment was done to determine concentrations of acetic or propionic acid that allowed growth of *A. parasiticus* in a medium with an initial pH value of 4.5 or 5.5. At an initial pH of 5.5, maximum concentrations that allowed growth were 1% acetic acid or 0.25% propionic acid. When the initial pH was 4.5, the values were 0.25% for acetic acid and 0.10% for propionic acid. Based on these results, concentrations of acid chosen for further experiments with the medium at pH 5.5 were 0.0, 0.25, 0.5, 0.75 and 1.0% for acetic and 0.05, 0.1, 0.15, 0.2 and 0.25% for propionic acid. When the initial pH of the medium was 4.5, concentrations of acetic acid used were 0.0, 0.1, 0.15 and 0.25% and those of propionic were 0.0, 0.05 and 0.1%. A glucose-yeast extract-salts medium described by Yousef and Marth *(34)* served for preliminary and later experiments.

A. parasiticus NRRL 2999 was obtained from the Northern Regional Research Center, U.S.D.A., Peoria, IL. The mold was grown at 28°C on slants of Mycological agar. After 7 d, spores were harvested by adding sterile distilled water and glass beads to cultures; glass beads helped to dislodge spores from the mycelium when shaken. The spore suspensions from several slants were pooled in a sterile 125-ml Erlenmeyer flask. The number of spores present per milliliter was determined by the plate count method using Mycological agar and incubation at 28°C.

Glucose-yeast extract-salts medium (283 ml) was dispensed into each of a series of 500-ml Erlenmeyer flasks and autoclaved at 121°C for 15 min. Following autoclaving, suitable amounts of 100% (w/v) acetic or propionic acid (Baker) were added to the medium to obtain the desired concentrations. Twelve milliliters of a spore suspension containing ca. 10^6 conidia per milliliter was added to each flask and the pH of the medium was adjusted to 4.5 or 5.5 by aseptically adding 10 *N* HCl or 40% NaOH. The medium was then dispensed into sterile 125-ml Erlenmeyer flasks. Each flask contained 25 ml of medium and ca. 10^6 conidia.

After 3, 7, and 10 d, contents of two flasks per concentration of acid were analyzed for pH, mycelium dry weight and aflatoxins B_1 and G_1; methods were previously described *(25)*. Analysis of variance was done on data using the Minitab statistical package and an IBM personal computer.

RESULTS AND DISCUSSION

pH of medium containing acetic or propionic acid and with an initial pH of 4.5 or 5.5

Acetic acid. At 3 d of incubation (Table 1), there was a decrease in pH of cultures without acetic acid, but an increase in pH of cultures in the presence of 0.50 or 0.75% acetic acid. No change in pH was observed for cultures in the presence of 0.25 or 1% acetic acid. The decrease in pH in cultures without acetic acid probably resulted from acids produced by the mold during growth. These results suggest that acetic acid at the concentrations used may have acted as a buffer and resisted the change in pH during mold growth. We made a similar observation when we evaluated lactic acid *(12)*.

When the initial pH of the medium was 4.5 (Table 2), there was a decrease in pH of all cultures except the one with 0.25% acetic acid, where a slight increase in pH occurred. This increase might be the result of limited metabolic activity of the conidia as no visible mycelium was observed. The rate of decrease in pH decreased with an increasing concentration of acetic acid. This can be attributed to decreased mycelial growth.

At 7 d of incubation, there was an increase in pH of all cultures except those with 1% acetic acid and an initial pH of 5.5 and 0.25% and an initial pH of 4.5 where

TABLE 1. *The pH of cultures of* A. parasiticus *containing various concentrations of acetic or propionic acid and incubated at 28°C; initial pH of medium was 5.5.*

	Incubation (d)		
Acid	3	7	10
Acetic (%)			
0	3.68	6.50	7.09
0.25	5.49	6.74	7.19
0.5	5.92	6.27	6.91
0.75	6.17	6.72	7.01
1.0	5.60	7.01	6.77
Propionic (%)			
0	2.70	6.81	7.12
0.05	2.88	6.73	6.99
0.1	3.67	6.94	7.31
0.15	4.43	7.13	7.43
0.2	4.90	7.10	7.55
0.25	5.43	6.73	7.40

TABLE 2. *The pH of cultures of* A. parasiticus *containing various concentrations of acetic or propionic acid and incubated at 28°C; initial pH of medium was 4.5.*

Acid	Incubation (d)		
	3	7	10
Acetic (%)			
0	2.82	5.68	6.24
0.1	3.19	6.70	7.00
0.15	3.46	6.72	6.97
0.25	4.62	3.49	3.06
Propionic (%)			
0.05	3.79	6.55	6.92
0.1	4.54	2.66	6.01

TABLE 3. *Dry weight of mycelium (g/25 ml of medium) produced by* A. parasiticus *in cultures containing various concentrations of acetic or propionic acid and incubated at 28°C, initial pH of medium was 5.5.*

Acid	Incubation (d)		
	3	7	10
Acetic (%)			
0	0.68	0.65	0.43
0.25	0.61	0.54	0.43
0.50	0.57	0.77	0.62
0.75	0.58	0.61	0.47
1.00	NG[a]	0.74	0.61
Propionic (%)			
0	0.65	0.51	0.47
0.05	0.61	0.57	0.52
0.1	0.45	0.57	0.49
0.15	0.35	0.61	0.47
0.2	0.30	0.56	0.45
0.25	0.08	0.64	0.51

[a]NG = No growth

TABLE 4. *Dry weight of mycelium (g/25 ml of medium) produced by* A. parasiticus *in cultures containing various concentrations of acetic or propionic acid and incubated at 28°C; initial pH of medium was 4.5.*

Acid	Incubation (d)		
	3	7	10
Acetic (%)			
0	0.62	0.69	0.55
0.1	0.47	0.55	0.46
0.15	0.39	0.54	0.49
0.25	NG[a]	0.61	0.69
Propionic (%)			
0.05	0.23	0.54	0.47
0.1	NG	0.65	0.73

[a]NG = No growth

decreases in pH were observed. The decrease in pH resulted from growth even though there was a lag in growth of the mold in these cultures. At 10 d of incubation, there was a further increase in pH of all cultures either with or without acetic acid in the medium at initial pH values of 4.5 or 5.5. The increase in pH at 7 d of incubation may have resulted from elevated levels of nitrogen in the medium *(7)* or autolysis of fungal cells *(34)*.

Propionic acid. At 3 d of incubation, the pH of the medium containing various concentrations of propionic acid decreased. The rate of decrease of pH decreased with increasing concentrations of propionic acid; this resulted from slow growth of mold. This change in pH was different from that occurring with acetic acid, suggesting that propionic acid did not act as a buffer. A decrease in pH was also observed in cultures growing in the medium containing 0.05% propionic acid and with an initial pH of 4.5; there was no change in pH of the medium containing 0.1% propionic acid as there was no mold growth.

At 7 d of incubation, there was an increase in pH of all cultures growing at all concentrations of propionic acid in the medium with an initial pH of 5.5; a further increase in pH was observed in these cultures at 10 d of incubation. A similar trend was noted for cultures in the medium containing 0.05% propionic acid and with an initial pH of 4.5, but this was not true for cultures in the presence of 0.1% propionic acid as a decrease in pH was observed first and then the pH had increased at 10 d of incubation.

Mycelium dry weight

Acetic acid. The maximum concentration of acetic acid that allowed growth of *A. parasiticus* was 1 or 0.25%, respectively, when the medium had an initial pH of 5.5 or 4.5. Acetic acid concentrations up to 0.75% did not inhibit mold growth (Table 3). At 3 d of incubation, there was no significant difference ($p>0.05$) in mycelial weight of cultures in the medium containing 0.0, 0.25, 0.5 or 0.75% acetic acid. There was no mold growth in the medium containing 1% acetic acid. Cultures in the medium with an initial pH of 4.5 (Table 4) produced less mycelial weight ($p>0.05$) as the concentration of acetic acid increased. There was no significant difference ($p>0.05$) in mycelial weight of cultures growing in the medium containing 0.1 and 0.15% acetic acid.

At 7 d of incubation, a slight ($p<0.05$) increase in mycelial dry weight was observed for cultures in the medium containing 0.50% acetic acid and with an initial pH of 5.5; there was no change ($p>0.05$) in mycelium dry weight of cultures in the presence of 0, 0.25 or 0.75% acetic acid. Cultures with 1% acetic acid overcame the initial inhibitory effect and then produced substantial amounts of mycelium. Cultures with 0.0 or 0.1% acetic acid exhibited a slight increase in mycelium dry weight, which was not significant ($p>0.05$), whereas cultures with 0.15 or 0.25% acetic acid showed a significant ($p<0.05$) increase in mycelial weight (Table 4).

At 10 d of incubation, cultures with or without acetic acid in the medium at an initial pH of 5.5 exhibited a slight decrease ($p<0.05$) in mycelium dry weight. This was also true for cultures in the medium with an initial pH of 4.5, except when 0.25% acetic acid was present which prompted a slight but not significant ($p>0.05$) in-

TABLE 5. *Accumulation of aflatoxin B_1 and G_1 (μg/25 ml of medium) at 28°C in a medium with various concentrations of acetic or propionic acid; initial pH of medium was 5.5.*

	3 d		7 d		10 d	
Acid	B_1	G_1	B_1	G_1	B_1	G_1
Acetic (%)						
0	783.7	5665.3	1082.2	8485.5	691.1	6470.2
0.25	395.8	3146.2	503.4	3812.3	264.2	4217.8
0.5	293.9	2442.3	367.0	4729.0	223.6	4063.2
0.75	270.4	1857.7	346.1	3895.7	213.4	3268.3
1.0	ND[a]	ND	306.2	3374.9	365.9	5056.9
Propionic (%)						
0	532.0	4196.2	625.0	5081.0	684.5	3644.8
0.05	324.1	2893.1	509.0	4439.0	327.0	3938.1
0.1	173.6	1325.5	430.6	3688.2	232.3	2371.1
0.15	110.0	806.1	347.2	2566.3	209.9	3031.2
0.2	95.5	634.3	399.5	2893.5	326.5	3537.6
0.25	22.4	124.1	462.9	3953.2	408.1	5274.2

[a]None detected

TABLE 6. *Accumulation of aflatoxin B_1 and G_1 (μg/25 ml of medium) at 28°C in a medium with various concentrations of acetic or propionic acid; initial pH of medium was 4.5.*

	3 d		7 d		10 d	
Acid	B_1	G_1	B_1	G_1	B_1	G_1
Acetic (%)						
0	571.6	4929.3	956.6	6688.0	840.9	5576.8
0.1	187.1	1135.8	378.3	3272.4	202.5	1776.9
0.15	226.2	1356.0	492.5	4467.7	441.2	3805.8
0.25	ND[a]	ND	604.2	3590.6	759.8	5134.2
Propionic acid (%)						
0.05	61.5	421.8	243.9	3179.0	161.1	1982.4
0.1	ND	ND	763.9	4969.1	862.8	8189.9

[a]None detected.

crease in mycelial weight. This decrease in mycelial dry weight may have resulted from autolysis of the mycelium with loss of intracellular solutes during filtration; onset of autolysis is usually associated with cessation of growth and onset of sporulation *(34)*.

Propionic acid. After 3 d of incubation there was no significant difference ($p>0.05$) in mycelium dry weight of cultures growing in the presence of 0.0 or 0.05% propionic acid (Table 3). Concentrations of propionic acid at and above 0.1% were detrimental to mold growth, as shown by low mycelium dry weight. There was no significant difference ($p>0.05$) in mycelium dry weight of cultures growing in the presence of 0.1 or 0.15% propionic acid. There was only slight mold growth in the presence of 0.25% and complete inhibition at 0.3% propionic acid. Results in Table 4 indicate that pH 4.5 potentiated the effect of propionic acid as the amount of mycelium produced was much less in the presence of 0.05% propionic acid at pH 4.5 than at pH 5.5. Moreover, there was no growth in the presence of 0.1% propionic acid at 3 d of incubation.

At 7 d of incubation, there was a nonsignificant ($p>0.05$) decrease in mycelium dry weight of cultures growing in the presence of 0.0 and 0.05% propionic acid (Table 3) and a significant ($p<0.05$) increase in mycelium dry weight of cultures growing in the presence of 0.1, 0.15, 0.2 or 0.25% propionic acid. Similarly, there was also a significant ($p<0.05$) increase in mycelium dry weight produced by cultures in a medium with 0.05 or 0.10% propionic acid and at an initial pH of 4.5. The increase in mycelium dry weight at 7 d of incubation suggests that propionic acid extended the lag phase of the mold. At 10 d there was a decrease in mycelium dry weight for all cultures except those in the medium containing 0.10% propionic acid and at an initial pH of 4.5 where there was an increase in mycelium dry weight.

Production of aflatoxin

Acetic acid. Biosynthesis and accumulation of aflatoxin were influenced most by concentration of acetic or propionic acid, initial pH of the medium and extent of mycelial growth (Tables 5, 6). At 3 d of incubation, cultures initially at pH 5.5 and with acetic acid produced decreasing amounts of aflatoxin G_1 as the amount of acetic acid increased. Cultures without acetic acid produced significantly ($p<0.05$) more aflatoxin G_1 than did cultures with 0.25% acetic acid. There was no significant ($p>0.05$)

difference in amounts of aflatoxin G_1 produced by cultures with 0.25 or 0.50% or with 0.50 or 0.75% acetic acid. However, there was a significant difference ($p< 0.05$) in amount of aflatoxin G_1 produced by cultures growing in the presence of 0.25 or 0.75% acetic acid (Table 5). Results also suggest that although acetic acid did not affect mycelial growth, it did affect production of aflatoxin G_1. No aflatoxin G_1 appeared at 3 d in the medium initially at pH 5.5 and containing 1% acetic acid. Cultures in the medium initially at pH 4.5 and containing 0.1 or 0.15% acetic acid produced small amounts of aflatoxin G_1 when compared to those growing in the absence of acetic acid. Also, no growth or aflatoxin production occurred in the medium containing 0.25% acetic acid and with an initial pH of 4.5, although cultures growing in the medium containing 0.25% acetic acid and with an initial pH of 5.5 produced substantial amounts of aflatoxins B_1 and G_1. Trends in production of aflatoxin B_1 were similar to those for aflatoxin G_1. Amounts of aflatoxin B_1 produced were about 10 times less than those of aflatoxin G_1.

At 7 d of incubation, amounts of aflatoxin G_1 produced by cultures growing in the presence of 0.0, 0.50 or 0.75% acetic acid increased significantly ($p<0.05$), whereas amounts of aflatoxin B_1 did not ($p>0.05$), except for cultures growing in the absence of the acid where the increase in aflatoxin B_1 was significant ($p< 0.05$). Also, cultures in the presence of 1% acetic acid overcame the initial inhibition and then produced substantial amounts of aflatoxin B_1 and G_1. This was also true for cultures in a medium initially at pH 4.5 and containing 0.25% acetic acid. A significant ($p<0.05$) increase in aflatoxin B_1 and G_1 was also observed in cultures growing in a medium containing 0.1 or 0.15% acetic acid and with an initial pH value of 4.5 (Table 6).

At 10 d of incubation, there was a significant ($p<0.05$) decrease in amounts of aflatoxin B_1 and G_1 in cultures growing in the medium without acetic acid and with an initial pH value of 5.5. This was not true for cultures in the medium containing 0.50 or 0.75% acetic acid and with an initial pH value of 5.5 since there was no significant ($p>0.05$) change in amounts of aflatoxin B_1 and aflatoxin G_1 present. There was a significant ($p>0.05$) increase in amount of aflatoxin G_1 but not of aflatoxin B_1 in cultures growing in the presence of 1% acetic acid. This was also true when the medium contained 0.25% acetic acid and had an initial pH of 4.5 as there was a significant ($p<0.05$) increase in both aflatoxin B_1 and G_1. Amounts of aflatoxins B_1 and G_1 also decreased in cultures in the medium initially at pH 4.5 and containing 0.0, 0.1, or 0.15% acetic acid.

Buchanan and Ayres *(3)* reported that sodium acetate was inhibitory at an acid pH, and concentrations of 1 and 2 g/100 ml completely inhibited growth and aflatoxin production in a medium at an initial pH value of 4.5 or 5.0, respectively. Uraih and Chipley *(31)* found that maximum growth of and aflatoxin production by *A. flavus* occurred in a medium containing 0.2% sodium acetate and with a pH value of 4.5. They further observed that the mold grew and produced aflatoxin in the presence of 2% sodium acetate. Our results differ from those of Buchanan and Ayres *(3)* and Uraih and Chipley *(31)* probably because acetic acid is more effective in inhibiting mold growth and aflatoxin production than is sodium acetate.

Propionic acid. Accumulation of aflatoxin B_1 and G_1 decreased with an increasing concentration of propionic acid in the medium with an initial pH value of 5.5 (Table 5) or 4.5 (Table 6) at 3 d of incubation. Cultures in the medium initially at pH 5.5 and containing 0.05% propionic acid produced significantly ($p<0.05$) less aflatoxin B_1 and G_1 than appeared in the control; however, at the same time these cultures produced significantly ($p<0.05$) more aflatoxin B_1 and G_1 than did those in the medium initially at pH 5.5 and containing 0.1, 0.15, 0.2 or 0.25% propionic acid. There was no significant difference ($p>0.05$) in amounts of aflatoxin B_1 and G_1 produced by cultures in the presence of 0.15 or 0.25% propionic acid in the medium with an initial pH of 5.5. Cultures in the medium containing 0.25% propionic acid with an initial pH of 5.5 produced significantly ($p<0.05$) less aflatoxin B_1 and G_1 than did those growing in the medium with 0.2% propionic acid and with an initial pH of 5.5.

There was no growth in the medium initially at pH 4.5 and containing 0.1% propionic acid, whereas only small amounts of aflatoxin B_1 and G_1 were produced by cultures in the presence of 0.05% propionic acid.

At 7 d of incubation, amounts of aflatoxin B_1 and G_1 produced by cultures growing in the medium initially at pH 5.5 and containing propionic acid increased significantly ($p<0.05$). Cultures growing in the presence of 0.25% propionic acid produced significantly ($p<0.05$) more aflatoxin G_1 than did those growing in the presence of 0.15 or 0.20% propionic acid, but there was no significant difference ($p>0.05$) in amounts of aflatoxin B_1 produced. When the medium contained 0.05 or 0.1% propionic acid and had an initial pH of 4.5, large amounts of aflatoxin B_1 and G_1 occurred at 7 d.

At 10 d of incubation amounts of aflatoxin B_1 and G_1 decreased in all cultures except in those in the medium containing 0.25% propionic acid and with an initial pH of 5.5 or in the medium containing 0.1% propionic acid and with an initial pH of 4.5 where there was an increase in aflatoxin B_1 and G_1. This ability of cultures to overcome initial inhibition and then produce large amounts of aflatoxins when growing in the presence of subinhibitory levels of preservatives has been reported by others *(5,11,13,31,34)*. The decrease, in amounts of aflatoxin B_1 and G_1 at 10 d of incubation has also been reported by others *(25,29,34,35)*. This decrease in all likelihood, is caused by degradation of the toxin through intracellular fungal enzymes that are liberated following autolysis of the mycelium *(10,34)*. The concentration of propionic acid in our work which completely inhibited growth and aflatoxin production is in general agreement with that reported by others *(3,22,30,31)*.

TABLE 7. *Proportions undissociated of acids at various pH values.*[a]

Acid	pK_a	Undissociated acid (%) at pH						
		3.0	3.5	4.0	4.5	5.0	5.5	6.0
Acetic	4.75	98	95	85	64	36	15	5.4
Propionic	4.88	99	96	88	71	43	19	7.0

[a]According to Lueck *(21)*.

Table 7 gives the percent of undissociated acetic and propionic acid at different pH values. At pH 5.5 and 1% acetic acid, the amount of undissociated acetic acid is 150 mg, whereas at pH 4.5 and 0.25% acetic acid, the amount of undissociated acid present if 160 mg. Similarly for propionic acid, at pH 5.5 and a concentration of 0.25%, the amount of undissociated acid present is 47.5 mg and at pH 4.5 with a concentration of 0.1%, the amount of undissociated propionic acid present is 71 mg. This explains why a concentration of 1% acetic acid is needed at pH 5.5, whereas at pH 4.5, 0.25% acetic acid is sufficient to have the same effect. This is also true for propionic acid.

Results of this study suggest that propionic acid is more effective in inhibiting growth and aflatoxin production by *A. parasiticus* than is acetic acid when low concentrations of an additive are considered. Moreover, our results also indicate that by lowering the pH, effects of acetic or propionic acid can be potentiated at lower concentrations.

ACKNOWLEDGMENTS

Research supported by the College of Agriculture and Life Sciences, University of Wisconsin-Madison, by the American-Mideast Educational and Training Services through a Peace Fellowship to Fathy E. El-Gazzar and by the Universiti Pertanian Malaysia through support of Gulam Rusul.

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from chilled meat (5.47) was significantly lower than the count (5.92) of the product from the hot meat, but the difference was small. Similarly, APC_{35} did not differ greatly between chilled or hot meat.

This evaluation of the microbiological condition of hot-boned beef primals and ground meat products, from USDA Cutter and Canner carcasses, under commercial conditions, did not demonstrate inordinate bacterial populations as suggested by Cuthbertson *(2)*. Rather, hot boning of beef can yield meat and meat products of good microbiological quality.

ACKNOWLEDGMENTS

We thank Mr. E. J. Koch and Dr. L. Douglass for advice and assistance in analyzing the data and Mr. N. Dvorak of Packerland International, Inc., Greenbay, WI, for his cooperation and use of facilities, and Dairilab Service, Manitowoc, WI for cooperation in the microbiological evaluation of the meat.

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