Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: https://www.tandfonline.com/loi/tbeq20

The Evaluation of Antifungal Effect of Calcium Propionate on Different Mold Species

M. Birbir & N. Çakırlı Doğu

To cite this article: M. Birbir & N. Çakırlı Doğu (2003) The Evaluation of Antifungal Effect of Calcium Propionate on Different Mold Species, Biotechnology & Biotechnological Equipment, 17:1, 74-80, DOI: 10.1080/13102818.2003.10819198

To link to this article: https://doi.org/10.1080/13102818.2003.10819198

THE EVALUATION OF ANTIFUNGAL EFFECT OF CALCIUM PROPIONATE ON DIFFERENT MOLD SPECIES

M. Birbir[1], N. Çakırlı Doğu[2]
Marmara University, Science and Arts Faculty, Department of Biology, Division of Plant Diseases and Microbiology, Göztepe, İstanbul, Turkey[1]
Bureau Veritas Gözetim Hizmetleri Limited Şirketi, Bayar Caddesi G-6 Sokak No: 8 Tema İş Merkezi, Kozyatağı, İstanbul, Turkey[2]

ABSTRACT

The inhibitory effect of calcium propionate, on growth of Aspergillus flavus, Aspergillus versicolor, Aspergillus niger, Penicillium citrinum, Penicillium verrucosum, Cladosporium macrocarpum, Fusarium semitectum, Mucor racemosus and Rhizopus oligosporus was evaluated at pH 5, 6 and 7 on Potato Dextrose Agar. The highest inhibition of the mold species was seen at pH 5 on the medium. The inhibitory effect of calcium propionate on the mold colonies increased significantly when the concentration of calcium propionate was raised from 1g/l to 2g/l. The inhibitory effect of calcium propionate was the highest at a concentration of 3g/ to 4g/l. 1g/l, 2.5g/l and 4g/l of calcium propionate were added to sterilized ground corn with 14% humidity to determine the effect of calcium propionate on the different molds growth at 25 °C. The colony counts of mold species decreased from 10^6 cfu/ml to 10^5 cfu/ml at concentrations of 2.5 g/l and 4 g/l calcium propionate in PDA at pH 5. All concentrations of calcium propionate retarded mold growth in corn samples during a span of 110 days storage whereas the number of mold colonies on corn did not change significantly after 110 days of storage. It was determined that tested levels of calcium propionate were insufficient for the inhibition of different mold growths.

Introduction

It is well known that when the conditions allow, mold can grow and spoil grain in the fields and during storage. Researchers have shown that the genera of mold that cause the most frequent contamination are *Aspergillus*, *Penicillium* followed by *Cladosporium*, *Helminthosporium*, *Mucor*, *Fusarium* and *Monilia* (1, 2, 3). If the contaminated grain with these molds are used in the preparation of food for consumption, these foods may contain mycotoxins. The major mycotoxin producing molds are members of the genera *Aspergillus*, *Penicillium* and *Fusarium*. Aflatoxins which are produced by toxigenic *A. flavus* and *A.parasiticus* have been shown to be hepatotoxic, hepatocarcinogenic, nephrotoxic and teratogenic leading to cramps, strokes, imbalance, restriction of growth, slowing of the immune system and causing stomach, lung, salivary glands, colon and skin cancers (4).

Vesonder and Horn (5) have shown that *A. versicolor* found in stored grain can produce sterigmatocystin which has a carcinogenic potential. In 1952, 25 people became ill with nausea and vomiting in Tokyo, following consumption of corn contaminated with *Fusarium* species (6). In other studies, *P. citrinum* that can grow on corn, wheat, oats, rye and rice can produce a mycotoxin called citrinin and this can lead to kidney lesions. *Cladosporium* mycotoxin can lead to alimentary tract diseases (7).

Antifungal agents have been used to reduce the growth of mycotoxigenic mold in cereal commodities. Only a few chemicals are legally acceptable for food preservation. Among the most effective are benzoic acid (0.1%), sorbic acid (0.2%), propionic acid (0.32%), parabens (0.1%) and sodium diacetate (0.32%). Sorbates, propionates, benzoates, butylated hydroxyanisole have been used as antifungal agents and it has been shown to have strong fungicidal activity (8, 9). Researchers have stated that especially propionic acid had the greatest antifungal activity. It is effective in reducing yeast and mold inocula which are responsible for aerobic deterioration in silages. The antifungal effect of propionic acid is enhanced as pH decline, making it an ideal candidate for improving the aerobic stability of corn silage where pH is low. Due to corrosive effect of propionic acid, the acid salts, e.g., calcium, sodium and ammonium propionate have become more widely used in commercial products (10).

The objective of this work was to study the inhibitory effects of different concentrations of calcium propionate on growth of *A. flavus*, *A. versicolor*, *P. verrucosum*, *P. citrinum*, *Cl. macrocarpum*, *F. semitectum*, *M. racemosus* and *R. oligosporus* at pH 5, 6 and 7 on Potato Dextrose Agar and on ground corn containing the tested mold species with 14 % moisture content.

Materials and Methods

Cultures of A. flavus, A. versicolor, P. verrucosum, P. citrinum, Cl. macrocarpum, *F. semitectum, M. racemosus* and *R. oligosporus* were obtained from Department of Biology, Uludağ University and Marmara University, Turkey. These mold species were isolated from corn samples in previous studies.

These mold species were streaked onto Potato Dextrose Agar (PDA, Oxoid, CM 139) and incubated for 7 days at 25 °C. Mold spores were generated by culturing each species on 100 ml PDA in an Erlenmeyer flask incubated at 25 °C for 7 days. Spores were harvested by washing the PDA surface with sterile Butterfield's buffered phosphate diluent's (APHA, 1992) containing 0.01% Triton X-100 (Merck 108603). The resulting spore suspension was filtered through several layers of sterile cheese cloth to remove any hyphal fragments. Then filtered spores were centrifuged at 4000 rpm and washed three times with sterile saline, centrifuging prior to each wash. The concentration of spores in the suspension was determined by viable spore count on PDA plates using the spread plate and surface count technique. Based on the counts, spore suspensions were adjusted to 10^6 spores/ml (11).

The study was done in three phases. For phase 1, PDA was used as a basal medium for all experiments. The medium was adjusted to pH 5, 6 and 7 using 1N NaOH and 1N HCl before sterilization at 121 °C for 15 minutes. A stock solution of sterile calcium propionate (Ets, Louis François S.A) was aseptically added to the flask containing the media (50 °C) to give final concentration of calcium propionate of 1 g/l, 1.5g/l, 2g/l, 2.5g/l, 3g/l, 3.5g/l and 4g/l. Also the pH of media were checked after adding calcium propionate. The medium without calcium propionate served as the control. Twenty milliliters of the medium was dispensed into sterile plates. Then PDA was spot inoculated with the appropriate spore suspension of each molds tested. All trials were done in triplicate. The inoculated plates were incubated for 7 days at 25 °C. The diameter of colonies were measured and the data recorded during 7 days of incubation period. The diameter of tested mold colonies were compared with colony diameter of control samples (12,13).

In the second phase of experiment, plate counts were done to quantitatively determine the effect of calcium propionate on mold growth. A stock solution of sterile calcium propionate was aseptically added

to the flask containing the media (50 °C) to give a final concentration of calcium propionate of 1 g/l, 2.5g/l and 4 g/l. Medium pH was adjusted to 5. Twenty milliliters of the medium was dispensed into sterile plates. The spore suspensions were adjusted to 10^6 spores/ml and serially diluted in sterile physiological saline solution. The diluted spore suspensions were plated on the medium containing 1 g/l, 2.5g/l and 4 g/l of calcium propionate. The inoculated plates were incubated at 25 °C, examined for growth everyday and counted 7 days later. Colony counts were then multiplied by the dilution factor.

In the third phase of experiment, ground corn was used as a substrate. Fifty grams of ground corn with a moisture content of 14% by weight were placed into a series of regular (450 ml) jars. 1g/kg, 2.5 g/kg and 4 g/kg concentration of calcium propionate were mixed with corn. The jars were capped loosely, autoclaved at 121 °C for 20 minutes and cooled. 1 ml of an aqueous suspension of tested mold spores, adjusted to 10^6 spores/ml was added to each jar. After inoculation, the jars were shaken for 3 minutes and incubated at 25 °C for 110 days. The jar lids were kept loose during storage. Triplicate jars were prepared for each calcium propionate concentration level. The jars were checked daily for visible signs of mold growth on corn. At the end of the incubation period the jars were opened under sterile conditions in a biological safety cabinet, ten grams of mixture were removed from the jars and added to sterile 90 ml of Butterfield's buffered phosphate diluent's in flasks. These mixtures were serially diluted in sterile physiological saline solution and plated on PDA. The plates were incubated at 25 °C, examined for growth everyday and counted 7 days later. Spores present in per milliliter was determined by plate count method. Colony counts were then multiplied by the dilution factor. Results were expressed as cfu/ml of original spore suspension (14).

Results and Discussion

The inhibitory effects of calcium propionate on growth of *A. flavus, A. versicolor, A. niger, P. verrucosum, P. citrinum, Cl. macrocarpum, F. semitectum, M. racemosus* and *R. oligosporus* were studied at three different pH levels and shown in **Fig. 1, 2 and 3.**

Increasing concentration of calcium propionate caused more inhibitory effects on the amount of total mycelium growth of tested mold species. Calcium propionate at 3.5 g/l and 4 g/l delayed mold growth of *A. flavus, A. versicolor, A. niger, P. verrucosum, P. citrinum Cl. macrocarpum, F. semitectum, M. racemosus and R. oligosporus* in Potato Dextrose Agar at pH 5 and 6. Especially at a concentration of 4 g/l, calcium propionate displayed the highest inhibitory effect on tested mold species. As the pH of the medium and the incubation periods of molds increase, inhibitory effect of calcium propionate decreased although the concentration of calcium propionate was increased. *A. niger, A. flavus, P. verrucosum* and *R. oligosporus* did seem to be slightly more susceptible to inhibition by high concentration of calcium propionate at pH 5 and 6. Tested mold species showed the highest inhibition at pH 5.

Sebti and Elaraki (12) explained that the maximum pH for antimicrobial activity was 5.5 for calcium propionate. Our result was similar to the authors results.

A. versicolor, P. citrinum, M. racemosus and *C. macrocarpum* did seem to be slightly resistant to inhibition by high concentration of calcium propionate at pH 5, 6 and 7. The inhibitory effects of calcium propionate against *Cl. macrocarpum, F. semitectum* and *M. racemosus* were completely lost after 4 days of incubation period at pH 5, 6 and 3 days of incubation period at pH 7. Results in Fig. 1, 2 and 3 show there was a net increase in mycelial growth at the end of 4 days of incubation in the presence of increasing amounts of calcium propionate, when compared to values

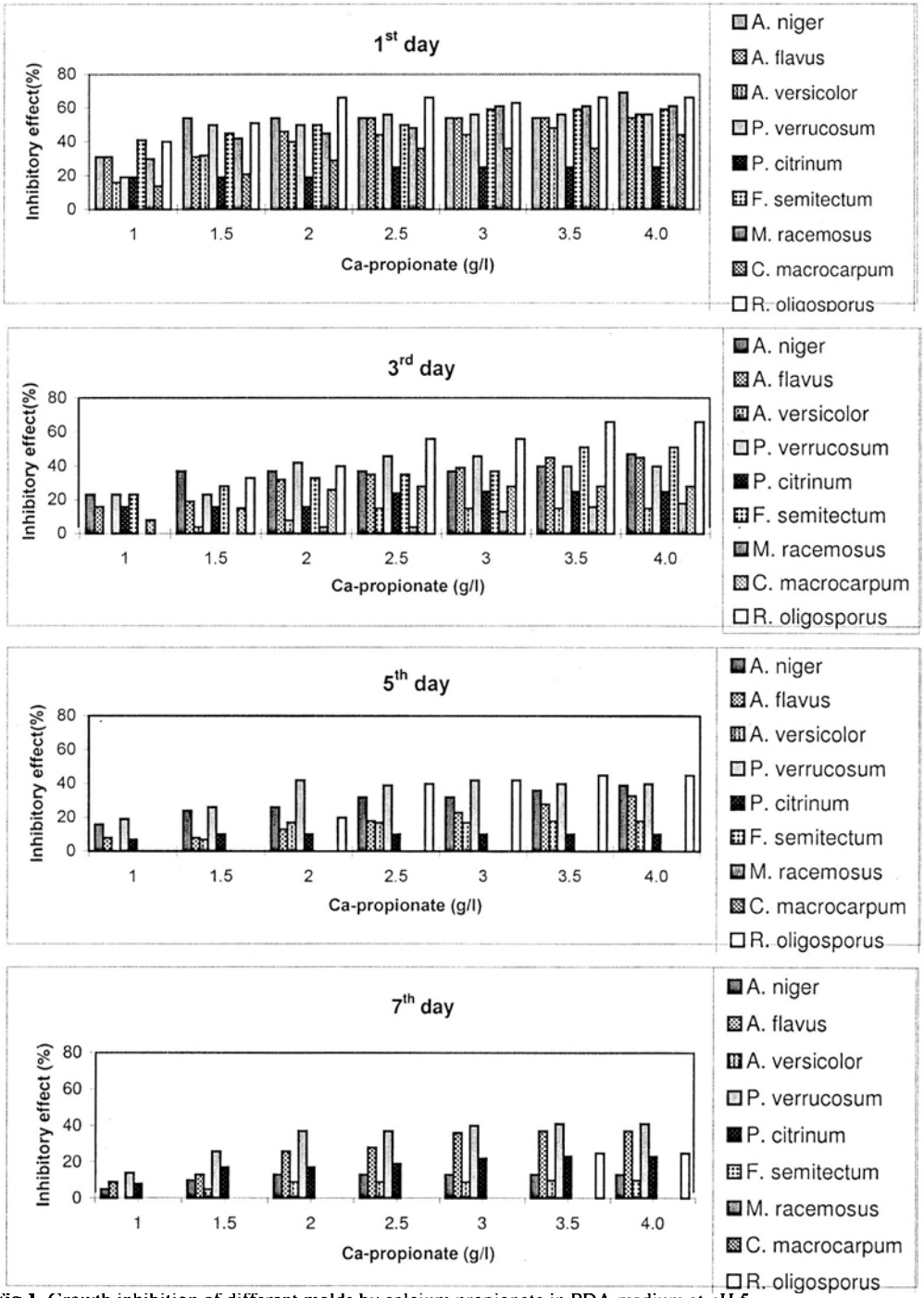

Fig.1. Growth inhibition of different molds by calcium propionate in PDA medium at pH 5.

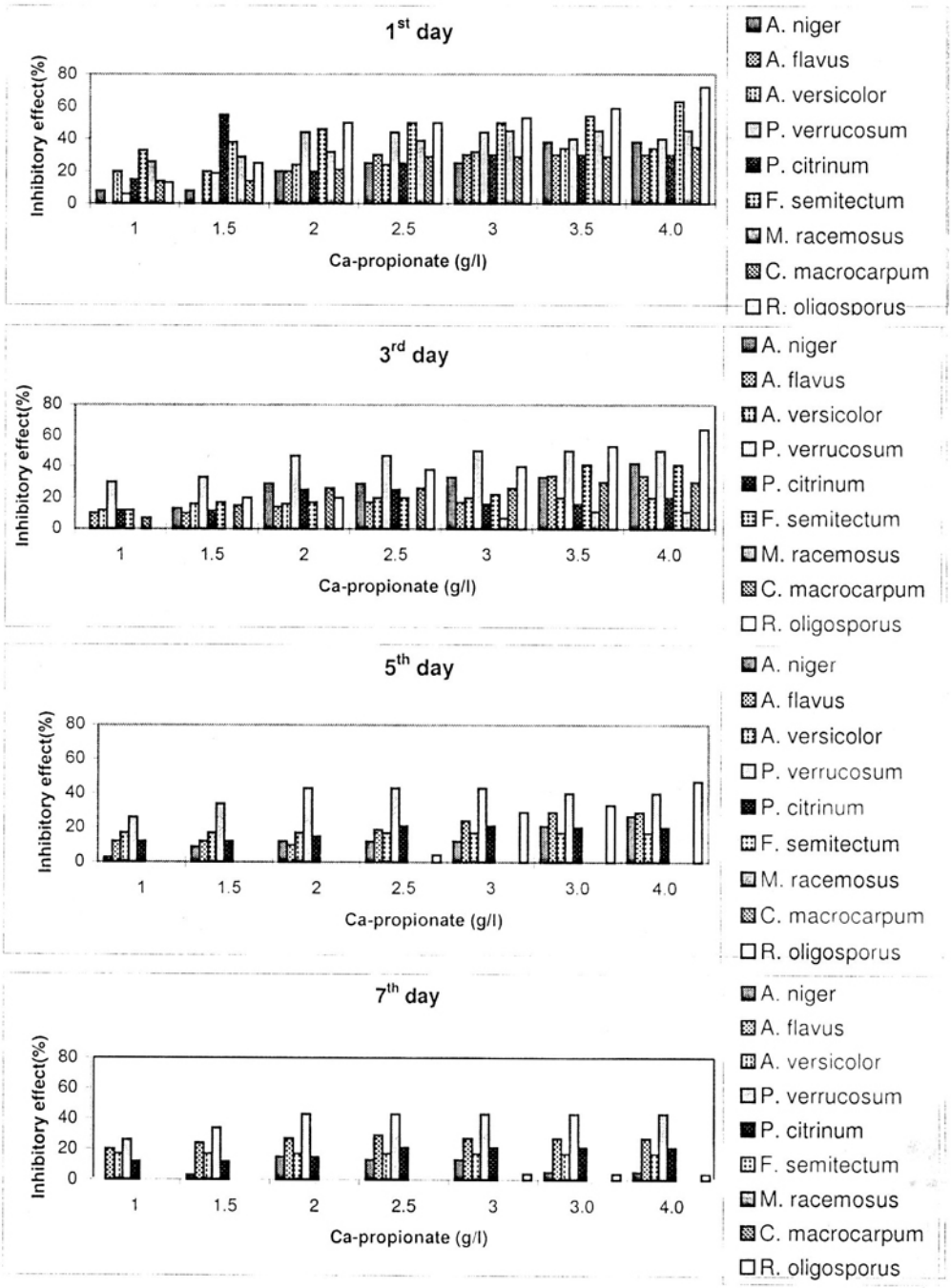

Fig.2. Growth inhibition of different molds by calcium propionate in PDA medium at pH 6.

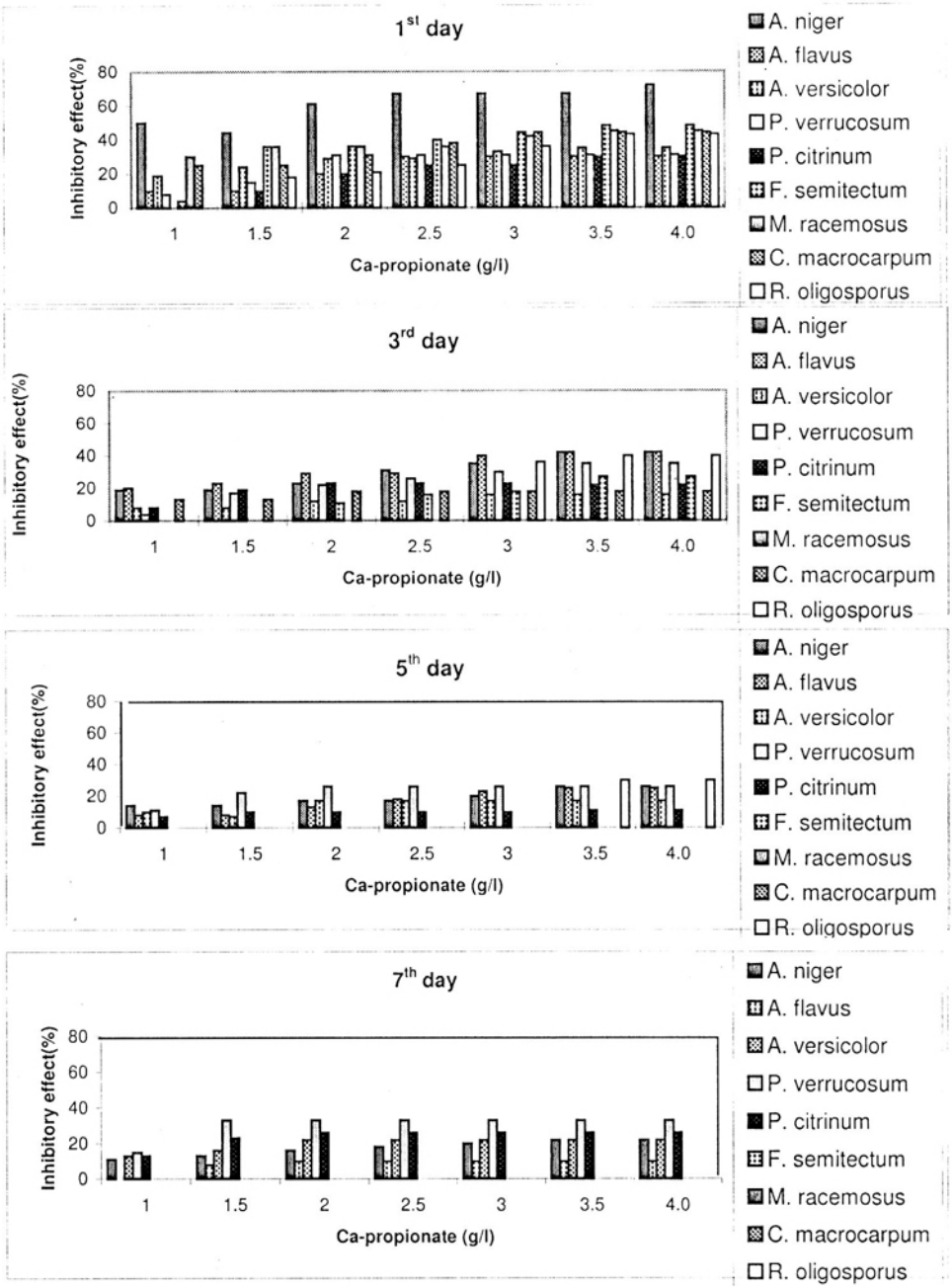

Fig.3. Growth inhibition of different molds by calcium propionate in PDA medium at pH 7.

obtained from the control.

Colony counts decreased from 10^6 cfu/ml to 10^5 cfu/ml at concentrations of 2.5 g/l and 4g/l of calcium propionate in PDA at

pH 5. However 1 g/l concentration of calcium propionate was not effective in reducing the spor concentration of tested mold species.

When the ground corn was treated with 1 g/l, 2.5 g/l and 4 g/l concentrations of calcium propionate, all concentrations of calcium propionate were almost equally effective on tested mold species at 25 °C for 110 days. Mold growth was not seen on corn samples during 110 days of storage. The number of mold spores on corn changed from 10^6 spores /ml to 10^5 spores/ml after 110 days of storage. It was determined that tested levels of calcium propionate were insufficient for the completely inhibition of mold growth.

Our results did not corroborate the findings of Sebti and Eleraki (12). The authors found that 3.2 g/kg of calcium propionate inhibited 97% of total mold which included all the molds studied. They have shown that the combination of sorbic acid at 1 g/kg with calcium propionate at 1.6 g/kg led to 65% mold inhibition in 7 days at 25 °C. They also showed that the combination of calcium propionate used at the highest concentration; i.e. 3.2 g/kg, with cinnamon water extract at 20g/kg cinnamon equivalent led to the inhibition of only 25% of total mold after 7 days at 25 °C.

Tellez et al. (15) have shown that when used in combination, potassium sorbate and calcium propionate were better in improving the shelf-life of corn tortillas than if used separately.

Ray and Bullerman (16) have shown that sorbic acid and its salt were the most effective of the three organic acids benzoic, propionic and sorbic over the widest range of conditions in preventing mold growth and mycotoxin production. Sebti and Elaraki (12) found that the combination of sorbic acid at 0.75 g/kg with benzoic acid at 1 g/kg were able to inhibit 100% of total molds studied.

In conclusion, the effects of calcium propionate on mold growth differed according to pH and to mold species. It is evident that calcium propionate at tested levels was not suitable to inhibit the growth of tested mold species. Thus, it is important that calcium propionate must be used in food products at a sufficient level or in combination with other compounds to inhibit mold growth.

REFERENCES

1. **Bauchat L.** (Ed.) (1978) Food and Beverage Mycology. Avian Publication Company Inc., Wesport, Connecticut, 397-443.

2. **Christensen C.M., Sauer D.B.** (1982) Microflora Storage of Cereals Grains and Their Products, Third Edition, American Association of Cereal Chemists, St .Paul, Mn, 544.

3. **Birbir M., Ilgaz A., Yurdun T., Çiloğlu F.** (1995) Pendik Vet. Mikrobiol Derg., **26(2)**, 163-174.

4. **Samson R.A., Hoekstra E.S., Frisvad J.C., Filtenborg O.** (2001) Introduction of Food and Airborne Fungi. Sixth Edition, Centraalbureau voor Schimmelcultures, Netherlands.

5. **Vesonder R.F., Horn B.W.** (1985) NRRC. Agricultural Research Service, **49**, 234-235.

6. **Kurata H.** (1968) J. Food Hyg. Soc. Jpn., **9**, 431-441.

7. **Watson D.** (1984) Chem. and Ind., **27**, 536-540.

8. **Depasuquale D.A., Nabarawy A.E., Rosen J.D., Montville T.J.** (1990) J.of Food Prot., **53**(4), 324-328.

9. **Prescott L.M., Harley J.P., Klein D.A.** (1993) Microbial Growth and Metabolism and Microorganisms and the Environment. In: *Microbiology* Second Edition; Wm. C. Brown Communications, Inc., IA, 176-179, 810-812..

10. **Kung L., Sheperd A.C., Smagala A.M., Enders K.M., Bessett C.A., Ranjit N.K., Glancey J.L.** (1998) J. Dairy Sci., **81**, 1322-1330.

11. **Karunaratre A.,Wezenberg E., Bullerman L.B.** (1990) J. Food Prot., **53**(3), 230- 236.

12. **Sebti F., Elaraki A.T.** (1994) Lebensm Wiss. U.Technol., **27**, 370-374.

13. **Thompson D.P.** (1994) J. Food Prot., **57**(2),133-135.

14. **Lebron C.L., Mollins R.A., Walker H.W., Kraft A.A., Stahr H.M.** (1989) J.Food Prot., **52**, 329-336.

15. **Tellez G.A., Acuff G.R., Vanderzent C., Rooney L.W., Wanisha R.D.** (1988) J. Food Prot., **51**, 945-948.

16. **Ray L.L., Bullerman L.B.** (1982) J. Food Prot., **42**, 953-963.