Research Article

SCREENING FOR ANTIFUNGAL ACTIVITY OF COMMERCIALLY AVAILABLE CHEMICAL FOOD PRESERVATIVES

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ABSTRACT

The objective of this study was to investigate antifungal activity of chemical food preservatives against food associated fungi isolated from bakery product and pickles. Acetic acid showed maximum antifungal activity against two isolates each of *Aspergillus. luchuensis, A. flavus, Rhizopus. stolonifer, Mucor* sp. (100%) followed by one isolate of *Penicillium. oxalicum* (66.6%) and minimum in *Scopulariopsis* sp. (60%). Lactic acid showed antifungal activity against two isolates each of *A. luchuensis* and *A. flavus* (50%), but it did not show any antifungal activity against other food-associated fungi. Benzoic acid produced 75 to 100% mycelial growth inhibition against all the selected food-associated fungi except one isolate each of *A. flavus* and *P. oxalicum.* Citric acid and sodium acetate were found to be inhibitory only against *Scopulariopsis* sp. with mycelial growth inhibition of 12.5% considered as nil activity. Hence, acetic acid is the most active chemical food preservative as compare with other test chemical preservatives in inhibiting the growth of food-associated fungi. No antifungal activity was observed in control. These findings indicated that acetic acid could be used to inhibit the growth of fungal food spoilage and food-borne pathogens and can be used to improve the safety of food products.

Keywords: Acetic acid, antifungal activity, chemical food preservative, food associated fungi and percent inhibition.

INTRODUCTION

Fungi are major plant and insect pathogens and profused growth of fungi on animal hosts produce the disease collectively called mycoses, while dietary, respiratory, dermal, and other exposures to toxic fungal metabolites produce the diseases collectively called mycotoxicoses. Mold spoilage accounts between 1 and 5% of product losses depending on the season, type of product being produced and the method of processing [1]. Mycotoxins are fungal metabolites that are present in a large part of the world food supply and bear potential threat to food safety [2]. *Aspergillus, Penicillium* and *Fusarium* are known to produce mycotoxins in foods that result to cause mycotoxicoses after ingestion [3]. Some mycotoxins are mutagenic and carcinogenic and some display specific organ toxicity. The major target organs for these toxins in human are liver, kidney, nervous system and endocrine system[4]. In addition to the economic losses, another concern is the possibility of the production of mycotoxins. The fungal spores are killed during baking and the airborne molds recontaminate the baked goods during the processing of bread such as cooling, slicing, wrapping and storage operations[5]. The most common spoilage molds isolated from bakery goods belong to the genera *Rhizopus, Mucor, Penicillium, Aspergillus, Monilia, Endomyces, Cladosporium* and *Fusarium*[6]. Several molds especially aspergilli and penicillia produce mycotoxins such as aflatoxin, ochratoxin, cyclopiazonic acid, sterigmatocystin, fumitremorgens, territrems, echinulin, tryptoquivalines, penitrime A, secalonic acid D, patulin and citrinin that exhibit a wide range of toxicities [7, 8]. Very little research has been done specifically on the inhibitory activity of organic acids against food-associated fungi. In lieu of the above justification, the present endeavor was to evaluate the antifungal activity of five chemical food preservatives against food-associated fungal isolates from bakery products and pickles.

MATERIALS AND METHODS

Evaluation of Chemical Food Preservatives for Their Antifungal Activity

Five chemical food preservatives namely sodium acetate, citric acid, benzoic acid, acetic acid and lactic acid (RANKEM) were chosen on the basis of their applications in bakery products (Jay *et al.,* 2005) and evaluated for their antifungal activity against food-associated fungi by poisoned food technique[9, 10]

Preparation of Fungal Inoculum

The stock suspensions of eleven food-associated fungal isolates (*Aspergillus luchuensis* I, *A. luchuensis* II, *A. flavus* I, *A. flavus* II, *Penicillium oxalicum* I, *Rhizopus stolonifer* I, *R. stolonifer* II, *Scopulariopsis* sp. I, *Scopulariopsis* sp. II, *Mucor* sp. I and *Mucor* sp. II) were standardized to 10^6spores/ml by spectrophotometrically at 530nm and were adjusted to 80 to 85% transmittance. The fungal inoculum (10^6spores/ml) was also determined by plate count on PDA followed by incubation at 25^0C for 7 days and observations made for visible growth of fungi at regular interval during the incubation period[11, 12]

Preparation of Stock Solutions

The stock solutions of chemical food preservatives, sodium acetate, citric acid, benzoic acid (1% w/v i.e., 0.1g chemical preservative dissolved in enough sterile distilled water to make the final volume 10ml), acetic acid and lactic acid (1% v/v i.e., 0.1ml chemical preservative dissolved in enough sterile distilled water to make the final volume 10ml) were prepared [13].

Poisoned Food Technique

In poisoned food technique, all the food-associated fungal isolates were inoculated on PDA plates in triplicates and incubated for 25^{0}C for 3 to 5 days, to obtain young, actively growing colonies of molds. 100µl of a food preservative was mixed with 15ml of cooled molten (45 to 50^{0}C) PDA and allowed to solidify at room temperature for thirty minutes. A mycelial disc 6mm diameter, cut out from periphery of 3 to 5 day old cultures, was aseptically inoculated onto the medium. A PDA plate with 100µl sterile distilled water was used as control[11, 12, 14]. The inoculated plates were incubated at 25^{0}C and colony diameter was measured and recorded after 7 days. Percentage of mycelial growth inhibition was calculated as below:

Percent mycelial growth inhibition =	Diameter of fungal colony (mean) in control – diameter of fungal colony (mean) in the presence of food preservative / Diameter of fungal colony (mean) in control	x 100

RESULTS AND DISCUSSION

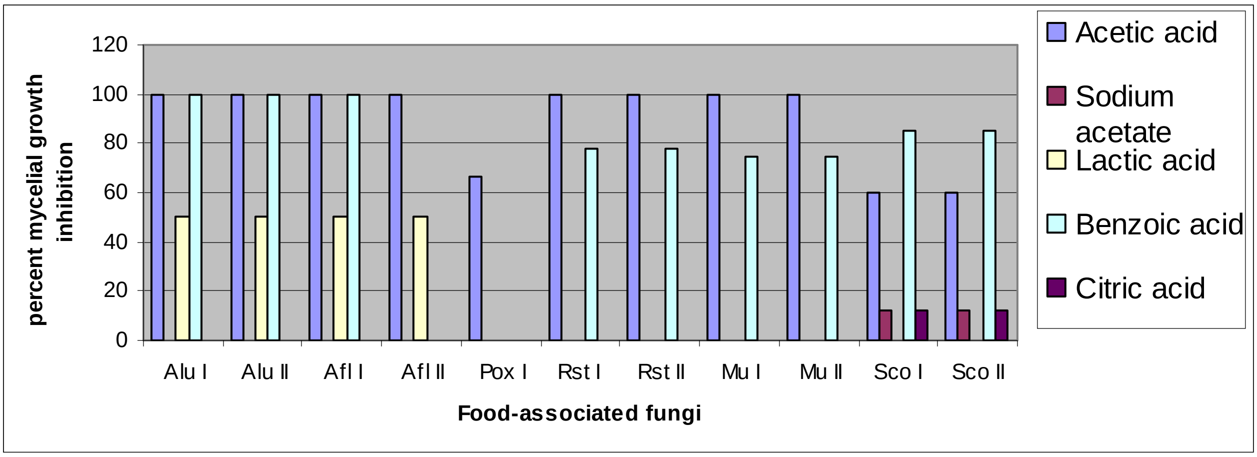
Of the five chemical food preservatives evaluated against fungi, acetic and benzoic acids have been found to be good inhibiting agents. Acetic acid showed antifungal activity against two isolates each of *Aspergillus luchuensis, A. flavus, Rhizopus stolonifer, Mucor* sp. and *Scopulariopsis* sp. and one *Penicillium oxalicum* isolate (Table 1 and Fig. 1).

Table 1: Antifungal activity of five chemical food preservatives against food-associated fungi by poisoned food technique.

Chemical food preservative (1%)	Percent mycelial growth inhibition										
	Alu I	Alu II	Afl I	Afl II	Pox I	Rst I	Rst II	Mu I	Mu II	Sco I	Sco II
Acetic acid	100	100	100	100	66.6	100	100	100	100	60	60
Sodium acetate	-	-	-	-	-	-	-	-	-	12.5	12.5
Lactic acid	50	50	50	50	-	-	-	-	-	-	-
Benzoic acid	100	100	100	-	-	77.7	77.7	75	75	85	85
Citric acid	-	-	-	-	-	-	-	-	-	12.5	12.5
Control (distilled water)	-	-	-	-	-	-	-	-	-	-	-

- No activity

Figure 1: Antifungal activity of five chemical food preservatives against food associated fungi by poisoned food technique.



Abbreviations: Alu I- *Aspergillus luchuensis* I, Alu II- *Aspergillus luchuensis* II, Afl I-*Aspergillus flavus* I, Afl II-*Aspergillus flavus* II, Pox I-*Penicillium oxalicum* I Rst I-*Rhizopus stolonifer* I, Rst II-*Rhizopus stolonifer* II, Mu I-*Mucor* sp. I, Mu II-*Mucor* sp. II, Sco I- *Scopulariopsis* sp. I, Sco II- *Scopulariopsis* sp. II.

Doyle *et al.*[15] reported the antifungal activity of acetic acid against *Aspergillus, Penicillium* and *Rhizopus* spp. and some strains of *Saccharomyces.* They also reported that molds and yeasts are more resistant to acetic acid than are bacteria. Lind *et al.*[16] who studied the effect of the three organic acids such as propionic acid, acetic and lactic acid, reported that propionic acid, followed by acetic and lactic acid as the most potent antifungal acid. Lactic acid has shown antifungal activity against two isolates each of *A. luchuensis* and *A. flavus* during the present study. Very little research has been done specifically on the antifungal activity of lactic acid against food-associated fungi [15].

Benzoic acid has been found to be the 2nd best antifungal agent. It produced 75 to 100% mycelial growth inhibition of eight food-associated fungi. According to Sofu *et al.*[17], its inhibitory action on yeasts and fungi is the background for a long term use of benzoic acid as the food-preservative. Our results are in agreement with Doughari *et al.* [13]who found that benzoic acid was most effective against aspergilli such as *Aspergillus niger, A. flavus* and *A. fumigatus* at 200mg/ml. Doyle *et al.* [15] reported the inhibitory concentration of benzoic acid at pH < 5.0 against most yeasts ranges from 20 to 700µl/ ml, while for molds it is 20 to 2000µl/ ml. Citric acid was found to be inhibitory only against *Scopulariopsis* sp. during the present study. They also reported that citric acid retards growth and toxin production by *Aspergillus parasiticus* and *A. versicolor* but not *Penicillium expansum.* The extent of antimicrobial activity of acetic and citric acids coincided with their degree of undissociation. Citric acid, with large dissociation constant was less detrimental to the tested microorganisms than was acetic acid. Moreover, weak acids are known to cause leakage of hydrogen ions across the cell membrane, acidifying the cell interior, and inhibiting nutrient transport. Some acids will dissociate to give ions such as lactate and acetate which the cell can transport and whose presence does not therefore inhibit energy yielding metabolite.

Furthermore it is known that the action of organic acids as antimicrobial agents is generally improved by anions which interfere with the dissociation of the acid molecules; certain specific cation may also singinficantly increase the effectiveness of organic acids by increasing the solubility of the acid in the microbial cell membrane (Leon *et al.*[18]. It can be concluded from the present study that acetic acid is more efficient antifungal agent than other organic acids tested and used it to reclaim the safety of bakery products and pickles.

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