Volume : 4 | Issue : 5 | May 2015 • ISSN No 2277 - 8179

Research Paper

Antimicrobial Activity of Preservatives Against Food Pathogens

Botany

KEYWORDS : Food spoilage, Preservatives, Pink rot, Penicillium, Aspergillus,Rhizopus, Alcaligenes, Pseudomonas, Xanthomonas, Bacillus, Streptococcus

Seema Rawat — Department of Botany and Microbiology, H.N.B Garhwal (Central) University, Srinagar, Uttarakhand, India.

Tripurari Prasad — Department of Biotechnology, Dolphin P.G. Institute of Biomedical & Natural Sciences, Dehradun, Uttarakhand.

ABSTRACT

Microbial spoilage of vegetables is generally described by the common term rot, along with the changes in the appearance, such as black rot, gray rot, pink rot, soft rot, stem-end rot refrigeration, vacuum or modified atmosphere packaging, freezing, drying, beat treatment, and chemical preservatives are used to reduce microbial spoilage of vegetables. The present research work was focussed on testing the antimicrobial potential of preservatives against the food spoilage microflora.Alcaligenes, Bacillus and Streptococcus were recovered from contaminated milk sample.Pseudomonas and Xanthomonas were isolated from spoiled orange and grape samples.Penicillim, Aspergillus and Rhizopus were isolated from spoiled cheese, pea, orange and grape samples.Acetic acid showed maximum antibacterial activity against Streptococcus and Bacillus. Citric acid showed maximum antibacterial activity against Alcaligenes, Pseudomonas and Xanthomonas. Acetic acid and Sodium nitrite showed maximum antifungal activity against Aspergillus, Penicillium and Rhizopus.

1. Introduction

Food spoilage can be defined as "any sensory change (tactile, visual, olfactory or flavour)"which the consumer considers to be unacceptable. Spoilage may occur at any stage along food chain. Spoilage may arise from insect damage, physical damage, and indigenous enzyme activity in the animal or plant tissue or by microbial infections (Gram*et al*., 2002).Most natural foods have a limited life. Perishable foods such as fish, meat and bread have a short life span. Other food can be kept for a considerably longer time but decomposes eventually. Enzymes can bring about destruction of polymers in some foods while chemical reactions such as oxidation and rancidity decompose others but the main single cause of food spoilage is invasion by microorganisms such as moulds, yeast and.

Food spoilage is caused by the growth of yeast and moulds. A furry growth covers the food and it becomes soft and often smells bad. Bacterial contamination is more dangerous because very often food does not look bad even though severely infected, it may appear quite normal. The presence of highly dangerous toxins and bacterial spores is often not detected until after an outbreak of food poisoning, laboratory examination uncovers the infecting agent.

Fresh vegetable are fairly rich in carbohydrates (5% or more), low in proteins (about 1 to 2%) and except for tomatoes, have high pH. Microorganisms grow more rapidly in damaged or cut vegetables(Mandrellet al., 2006). The presence of air, high humidity during storage increases the chances of spoilage. The common spoilage defects are caused by molds belonging to genera Penicillum, Phytophthora, Alternaria, Botrytis, andAspergillus. Among the bacterial genera, species from Pseudomonas, Erwinia, Bacillus and Clostridium are important (Bartz, 2006; Lindow and Brandl,2003).Bacterial spoilage associated with the souring of berries and figs has been attributed to the growth of lactic acid and acetic bacteria. To reduce spoilage, fruits and products are preserved by refrigeration, freezing, drying and reducing aw, vaccum packaging and heat treatment.

The aim of food preservation is to minimize the growth of microorganisms during the storage period, thus promoting longer shelf life and reduced hazard from eating the food. This work deals with characterization of food spoilage microorganisms and effect of preservatives upon their growth.

2. Methods

2.1 Collection of samples

The contaminated samples (Milk, oranges, grapes, cabbage, pea) were collected aseptically in sterile containers and were processed immediately in lab.

2.2Recovery of isolates

Isolates were recovered by enrichment in nutrient broth (bacterial cultures) and Potato dextrose broth (fungal cultures) and subsequent plating on Nutrient agar (NA) and Potato dextrose agar (PDA), respectively. Plates were incubated at 30±10C. All samples were plated in triplicates.

2.3Morphological characterization

Cell Morphology of bacterial isolates was studied by Gram staining and that of fungal culture by Lactophenol cotton blue staining.

2.4 Biochemical characterization

The various biochemical tests viz., Catalase test, Indole-Methyl Red –Voges-Proskauer-Citrate Utilization test (IMViC), Triple Sugar Iron (TSI) Test and Nitrate reduction tests were carried out according to Cappucino and Sherman(1992).

2.5 Antimicrobial activity of preservatives

Bacterial cultures were grown overnight in trypticase soy broth and their optical density was adjusted to 0.9-1.0 (range). These cultures were then inoculated in trypticase soy broth containing various preservatives (Sucrose, sodium chloride, sodium nitrite, acetic acid and citric acid) at conc. of 1%. Cultures were incubated in shaker at 30±10C, 200rpm. Growth of bacterial cultures was monitored at regular intervals by measuring absorbance of samples at 600nm.

Fungal culture discs were inoculated in sterile water blanks and vortexed for 10min. These cultures were inoculated in trypticase soy broth containing various preservatives (Sucrose, sodium chloride, sodium nitrite, acetic acid and citric acid) at conc. of 1%. Cultures were incubated in shaker at 30±10C, 200rpm. Growth of fungal cultures was monitored at regular intervals by measuring fresh weight and dry weight of fungal mycelium.

3. Results

3.1 Prevalence of food pathogens

A total of 5 bacterial isolates were recovered from spoiled samples which were identified based on morphological and biochemical characteristics 3 fungal isolates were recovered which were identified based on their colony characteristics and mycelium structure. *Alcaligenes, Bacillus* and*Streptococcus* were re-

Volume : 4 | Issue : 5 | May 2015 • ISSN No 2277 - 8179

covered from contaminated milk sample.*Pseudomonas* and*Xanthomonas* were isolated from spoiled orange and grape samples. *Penicillim*, *Aspergillus* and *Rhizopus* were isolated from spoiled cheese, pea, orange and grape samples.

3.2 Antimicrobial activity of preservatives

The various preservatives were tested for their antimicrobial activity against bacterial and fungal isolates recovered from spoiled milk, cheese, pea and fruit samples. Acetic acid and Citric acid were found to have pronounced antibacterial activity against all bacterial isolates (Fig. 1 to 5). Acetic acid showed maximum antibacterial activity against *Streptococcus* and *Bacillus*. Citric acid showed maximum antibacterial activity against *Alcaligenes, Pseudomonas* and *Xanthomonas*. Acetic acid and Sodium nitrite showed maximum antifungal activity against *Aspergillus, Penicillium* and *Rhicopus* (Fig. 6 to 8).

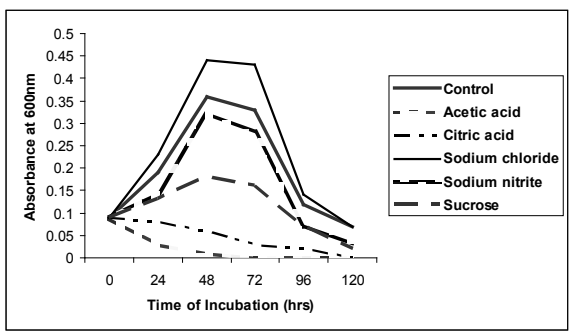

Fig.1: Monitoring of growth of *Streptococcus* in presence of various food preservatives

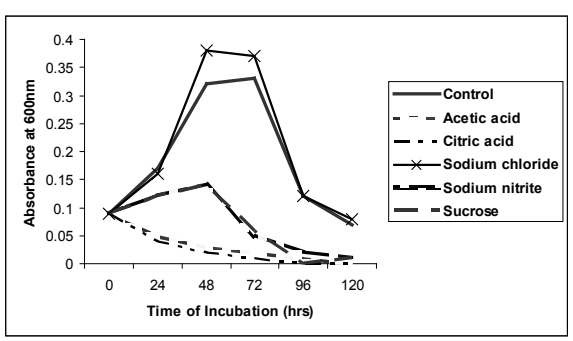

Fig.2: Monitoring of growth of *Alcaligenes* in presence of various food preservatives

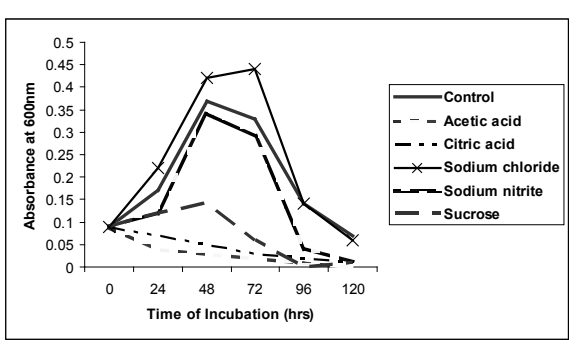

Fig.3: Monitoring of growth of *Bacillus* in presence of various food preservatives

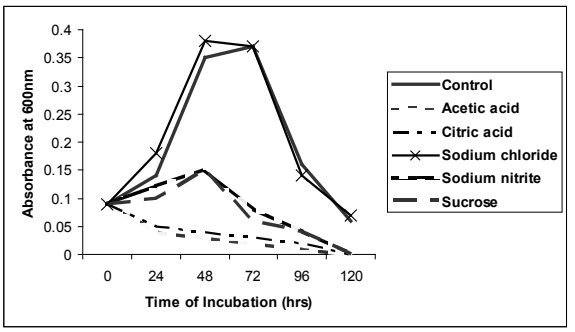

Fig.4: Monitoring of growth of*Pseudomonas* in presence of various food preservatives

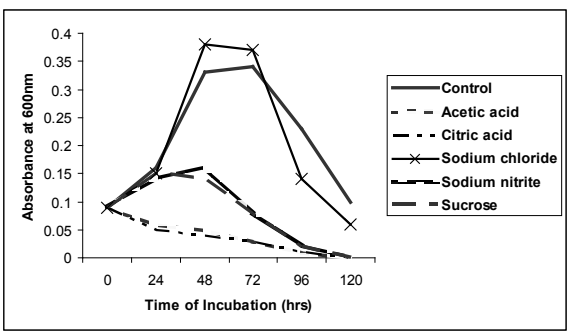

Fig.5: Monitoring of growth of*Xanthomonas* in presence of various food preservatives

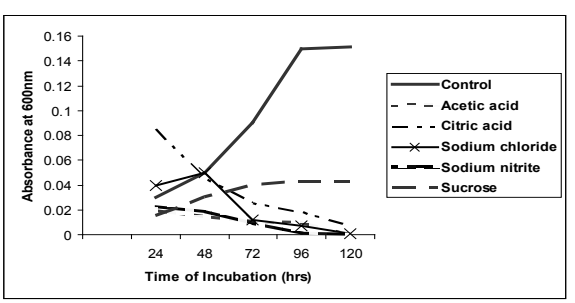

Fig.6a: Monitoring of growth of*Aspergillus*by estimation of fresh weight of mycelium at regular intervals in presence of various food preservatives

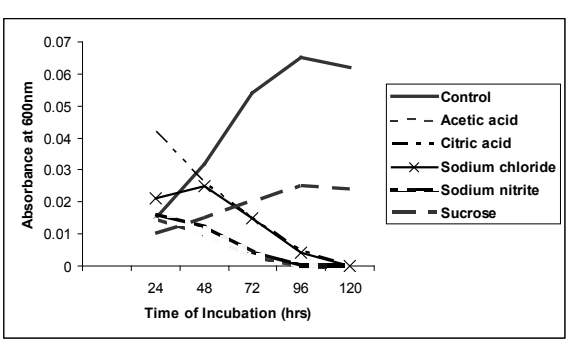

Fig. 6b: Monitoring of growth of*Aspergillus*by estimation of dry weight of mycelium at regular intervals in presence of various food preservatives

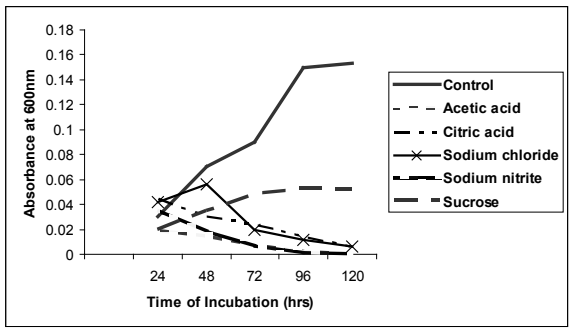

Fig.7a: Monitoring of growth of*Penicillium*by estimation of fresh weight of mycelium at regular intervals in presence of various food preservatives

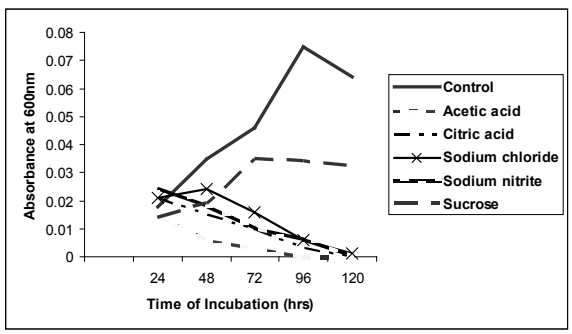

Fig.7b: Monitoring of growth of*Penicillium*by estimation of dry weight of mycelium at regular intervals in presence of various food preservatives

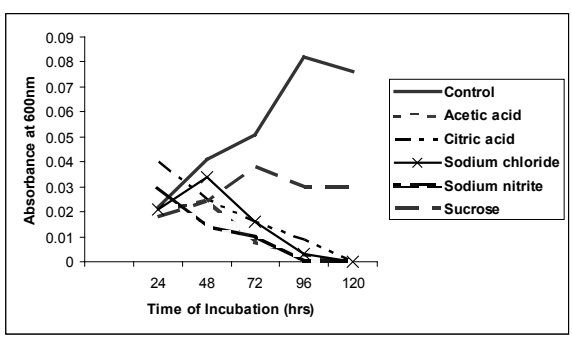

Fig.8a: Monitoring of growth of*Rhizopus*by estimation of fresh weight of mycelium at regular intervals in presence of various food preservatives

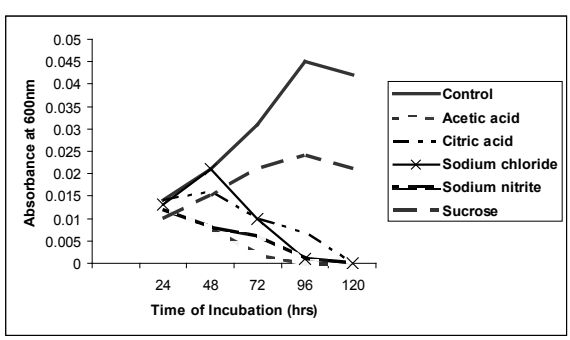

Fig.8b: Monitoring of growth of*Rhizopus*by estimation of dry weight of mycelium at regular intervals in presence of various food preservatives

4. Discussion

Fruits and vegetables are very important food commodities not only in India but all over the world. India, which is the second most populated country of the world, is still struggling to achieve self-sufficiency to feed about 800 million people. For this purpose, fruits and vegetables have got their specific importance to provide a balance and healthy diet to the people. India is the second largest producer of vegetables and fourth largest producer of fruits in the world. Though India is producing adequate quantities of fruits and vegetables, yet on account of losses in the field as well as in storage, they become inadequate. It is estimated one-fourth of the harvested fruits and vegetables is spoiled before consumption. Spoilage of fresh fruits and vegetables usually occurs during storage and transport. Mechanical damage may increase the susceptibility to decay and the growth of microorganisms may take place. Washing process in contaminated water may moisten surfaces enough to permit entry and growth of organisms. Storage in contaminated containers, use of contaminated dressing materials, possible contact with decayed products, unhygienic handling, fly infestation etc. will also cause an accelerated rate of spoilage.

In the present study *Pseudomonas* and *Xanthomonas* were isolated from spoiled orange and grape samples. *Pseudomonas* and *Xanthomonas* have been reported earlier also by Munsch*et al.* (2000). *Aspergillus* and *Rhizopus* were also recovered.These are common spoilage moulds.*Streptococcus*, *Alcaligenes*, *Bacillus* were isolated from spoiled milk samples in the present study. Milk is an excellent medium for growth for a variety of bacteria (Mayr*etal.,* 2004). Spoilage bacteria may originate on the farm from the environment or milking equipment or in processing plants from equipment, employees, or the air. Lactic acid bacteria (LAB) are usually thepredominant microbes in raw milk and proliferate if milk is not cooled adequately. When populations reach about 106cfu/ml, off-flavors develop in milk due to production of lactic acid and other compounds. Refrigeration suppresses growth of LAB and within one day psychrophilicbacteria (*Pseudomonas, Enterobacter, Alcaligenes*and some spore-formers) grow and can eventuallyproduce rancid odors through the action of lipases and bitter peptides from protease action (Dogan*et al.*, 2006).Pasteurization kills the psychrophiles and mesophilic bacteria (LAB), but heat-tolerant species (*Alcaligenes*,*Microbacterium,* and the sporeformers*Bacillus* and *Clostridium*) survive and may later cause spoilage in milk or other dairy products.However, post-pasteurization contamination of milk, particularly with *Pseudomonas* and some Gram-positive psychrophiles does occur(Stevenson *et al.*, 2003; Mayr*et al.*, 2004).

The aim of food preservation is to minimize the growth of microorganisms during the storage period, thus promoting longer shelf life and reduced hazard from eating the food. Chemical preservatives are substances which are added to food just to retard, inhibit or arrest the activity of microorganisms such as fermentation, putrefaction and decomposition of the food. Commonly used preservatives include, common salt, sugar, dextrose, spices, vinegar, ascorbic acid, benzoic acid and its salt, SO2 and the salts of sulphuric acid, nitrates, sorbic acid and its salts, propionic acid and its salts, lactic acid and its salts.Sugar, salts and acids are also used as food additives. Salts exert its preservative action by causing high osmotic pressure resulting in theplasmolysis of microbial cells, dehydrating food and microorganisms by tying up the moisture, ionizing to yield the chloride ion which is harmful to microorganisms andreducing the solubility of oxygen in water, sensitizing the cells against CO2. Sugar too causes plasmolysis of microbes. Acetic acid (Vinegar), Citric acid (Lime juice), Lactic acid (Lactose) etc. are also used. In the present study sodium chloride, acetic acid, citric acid, sodium nitrite and sucrose were used as preservatives. Acetic acid showed maximum antibacterial activity against Streptococcus and Bacil-

Volume : 4 | Issue : 5 | May 2015 • ISSN No 2277 - 8179

lus. Citric acid showed maximum antibacterial activity against Alcaligenes, Pseudomonas and Xanthomonas. Acetic acid and Sodium nitrite showed maximum antifungal activity against Aspergillus, Penicillium and Rhizopus.

REFERENCE

1. Bartz, J.A. 2006. Internalization and Infiltration. In G. M. Sapers, J. R. Gorney and A. E. Yousef (Eds.). Microbiology of fresh fruits and vegetables (pp. 75–94). New York: Taylor and Francis Group. | 2. Cappucino, J. and Sherman, N. 1992. Microbiology: A laboratory manual. Benjamin/Cummings Publishing Company, San Francisco. | 3. Dogan, B. and Boor, K.J. 2003. Genetic diversity and spoilage potentials among Pseudomonas spp. isolated from fluid milk products and dairy processing plants. Appl. Environ. Microbiol., 69: 130–138. | 4. Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B. and Givskov, M. 2002. Food spoilage—interactions between food spoilage bacteria. Int. J. Food Microbiol., 78: 79–97. | 5. Lindow, S. E. and Brandl, M. T. 2003. Minireview: Microbiology of the phyllosphere. Applied and Environmental Microbiology, 69: 1875–1883. | 6. Mandrell, R.E., Gorski, L., and Brandl, M.T. 2006. Attachment of microorganisms to fresh produce. In: G. M. Sapers, J. R. Gorney, and A. E. Yousef (Eds.), Microbiology of fresh fruits and vegetables (pp. 33–73). New York: Taylor and Francis Group. | 7. Mayr, R., Gutser, K., Busse, M. and Seiler, H. 2004.Gram positive non-sporeformingrecontaminants are frequent spoilage organisms of German retail ESL (extended shelf life) milk. Milchwissenschaft, 59: 262–266. | 8. Meyer, J.D., Cerveny, J.G. and Luchansky, J.B. 2004. Inhibition of nonproteolytic, psychrotrophic clostridia and anaerobic sporeformers by sodium diacetate and sodium lactate in cook-in-bag turkey breast. Int. J. Food Microbiol., 103: 37–45. | 9. Munsch, P., Geoffroy, V. A., Alatossava, T. and Meyer, J.M. 2000. Application of siderotyping for characterization of pseudomonas tolaasii and pseudomonas reactans isolates associated with brown blotch disease of cultivated mushrooms. Applied Environmental Microbiology, 66: 4834–4841. | 10. Stevenson, R.G., Rowe, M.T., Wisdom, G.B., and Kilpatrick, D. 2003. Growth kinetics and hydrolytic enzyme production of Pseudomonas spp. Isolated from pasteurized milk. J. Dairy Res., 70: 293–296.