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# Effect of various organic acids on the inhibition of Fresh cut vegetable spoilage bacteria by Agar well diffusion method

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**Abstract**

The fresh cut vegetables are easily spoiled by various pathogenic bacteria and make it unfit for long storage. Fresh cut produce is an important component of a healthy diet because it is a source of vitamins, minerals, fibre and antioxidants. The vegetables are the major dietary sources of nutrients of greater importance from the human nutritional point of view. It is also estimated that about 20 % vegetables produced is lost each year due to spoilage. Vegetables have been identified as significant sources of pathogens and chemical contaminants. The use of chemical preservatives and organic acids currently employed to control the fresh cut vegetables spoilage causing microorganisms including sulphites, sulphur dioxide, sodium chloride, phosphates, hydrogen peroxide, nitrates, nitrites, sodium diacetate, citric acid, acetic acid, lactic acid, benzoic acid, fumaric acid. The present study was undertaken to know about the effects of different concentrations of organic acids on the growth of the fresh cut vegetable spoilage bacterial strains were studied using Malic acid, Lactic acid and Citric acid. The increase in the concentration of organic acids shows increased inhibitory effect.

**Key words :** Vegetables, Spoilage, Preservatives, lactic acid.

Fresh cut produce is globally recognized as an important component of a healthy diet because it is a source of vitamins, minerals, fibre, and antioxidants. It is a fast-growing food category and an important component of a healthy diet. Fresh cut produce is a desirable food because the consumer perceives it as being healthy, tasty, convenient, and fresh, all of which are strong selling points to health-conscious and time-deprived consumers. Vegetables are the major dietary sources of nutrients of greater importance from the human nutritional point of view. Microbial food spoilage was characterized by the change in food products that renders it unacceptable to the consumer from a sensory

point of view. Spoilage of food stuffs may be caused by physical damage, chemical changes (oxidation and colour changes) or change in flavours and odours resulting from microbial growth and metabolism in the food products. Microbial growth in food substance is the most common cause of food spoilage and manifest itself as visible growth (slime, colonies), as textural changes (degradation of polymers) or as off-odours and off-flavours[1].

The growth and activity of food spoilage microorganisms is mostly described and studied as function of substrate base and of various parameters such as temperature, pH, water activity and atmospheric pressure. Food spoilage is one of the most important issues which were mainly faced by the food industries. In fact, food borne illnesses are a global problem, even in developing countries which is mainly caused by spore forming and toxin producing bacteria. Spoilage of fresh cut vegetable is predominantly caused by the microbial growth in variety of fresh cut product.Survival times for coliforms, bacterial pathogens and enteric viruses on most fresh produce are moisture- and temperature-dependent, and survival usually extends beyond the useful life of the product.It is also estimated that about 20% vegetables produced is lost each year due to spoilage. Vegetables have been identified as significant sources of pathogens and chemical contaminants[13]. Vegetables that are used as salad have been implicated as a cause of food poisoning and thus, they are hazardous to the health of the consumers who are infected with many types of disease. The use of chemical preservatives and organic acids currently employed to control the fresh cut vegetables spoilage causing microorganisms including sulphites, sulphur dioxide, sodium chloride, phosphates, hydrogen peroxide, nitrates, nitrites, sodium diacetate, citric acid, acetic acid, lactic acid, benzoic acid, fumaric acid. The focus has been on the microbiological safety through interventions strategies aimed at eliminating or reducing microbial hazards (human pathogens), mainly by using chemical disinfectants. The use of a chemical disinfectant in wash water provides a barrier to cross contamination of produce and is effective in removing disease-causing organisms from the surface minimally processed produce[3].

Natural preservatives are available from a variety of sources including plants, animals, bacteria, algae, and fungi. Microbial derived preservatives (*e.g.*, bacteriocin), plant derived preservatives (thyme essential oil, tea polyphenols, rosemary extract, *etc.*), and animal derived preservatives (*e.g.*, chitosan from crab or shrimp shells) have been demonstrated to have antimicrobial or antioxidant properties[8]. Organic acids, which have been used as preservatives, have also been investigated as sanitizers for application on vegetables to reduce populations of microorganisms. Citric acid has been reported to reduce *E. coli* O157:H7 and *L. monocytogenes* on lettuce leaves by 0.84 and 1.03 log, respectively[14].

Tohora Sultana *et al.,*[12] stated that organic acids are popular preservatives with marked anti bacterial traits, both acetic acid and vinegar exhibited and anti bacterial activity against Pseudomonas spp., however, both were initially found to harbour Pseudomonas spp., with a relatively higher bacterial load

especially in the vinegar samples.

Five different types of Fresh cut vegetables *viz*., Cabbage, Lettuce, Spinach, Broccoli and Cauliflower were collected for the present research from Cuddalore district, Tamil Nadu, India. The total bacterial population in collected fresh cut vegetables (Cabbage, Lettuce, Spinach, Broccoli and Cauliflower), was determined by Standard plate count (SPC) method. The collected fresh cut vegetables were serially diluted upto $10^{-6}$ dilution. One ml of samples was pipetted onto a sterile petridish with Nutrient agar. They were mixed well and allowed to solidify. Three replications were maintained and the uninoculated plates served as control. The dishes were incubated at 28±1°C and the colonies of bacteria appearing on the incubated plates were counted after 24 hours by using Qubec colony counter and expressed as $10^{-4}$ cfu/ml. Pour plate technique was used for the isolation of bacteria from the fresh cut vegetables collected from five different locations in Cuddalore district, Tamil Nadu, India. In this method, 1 ml of sample was thoroughly mixed with 99 ml of sterile distilled water, and then it was serially diluted by following standard procedure upto concentration of $10^{-6}$. Then, 1 ml of serially diluted samples from each concentration of samples were transferred to sterile petriplates and evenly distributed throughout the plates and sterile unsolidified Nutrient agar was poured and it was allowed to solidify. The Nutrient agar plates were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were isolated from the plates.The effects of different concentrations of organic acids on the growth of the fresh cut vegetable spoilage bacterial strains were studied using Malic acid, Lactic acid and Citric acid. The sterilized Nutrient broth was prepared and seeded with standard inoculum of the five bacterial strains, *Bacillus* spp, *Listeria* spp, *Escherichia* spp, *Salmonella* spp and *Pseudomonas* spp separately and plated. Different concentrations of malic acid, citric acid, and lactic acid *viz*.,0.5, 1.0, 1.5, 2.0 and 2.5% were prepared. Solution containing combinations of selected organic acids and hydrogen peroxide were prepared in a similar way by dissolving these chemicals in DI water to the final concentration of 1% for each component with 200ppm. Three replications were maintained in each treatment.

Hussain *et al.,*[10] reported that citric acid and lactic acid has reduced the growth and viability of *E. coli* and *S. typhymurium* load on fresh cut vegetables surface (22.60%, 20.10%) and (20.30%, 18.20%), respectively. The inhibitive effects of 5 % concentration were highest, followed by 3 % conc. least for 1 % conc. of both citric and lactic acid. The citric acid effects were significantly higher ($p<0.05$) than lactic acid at all concentrations (1.0, 3.0 and 5.0%).

The effect of Malic acid on the inhibition of growth of five freshcut vegetables spoilage bacterial strains *viz., Bacillus* spp., *Listeria* spp., *Escherchia* spp., *Salmonella* spp., and *Pseudomonas* spp. was studied and the results were presented in Table – 1. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of malic acid from 0.5 to 2.5 %. Highest zone of inhibition was recorded against *Listeria* spp. (25.22 mm), *Salmonella*

Table-1. Effect of Malic acid on the inhibition of Fresh cut vegetable spoilage bacteria by Agar well diffusion method

| Malic acid (conc)% | *Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | *Bacillus* spp | *Listeria* spp | *Escherichia* spp | *Salmonella* spp | *Pseudomonas* spp |
| Control | NZ | NZ | NZ | NZ | NZ |
| 0.5 | 13.50 | 16.31 | 12.29 | 15.71 | 11.30 |
| 1.0 | 15.26 | 18.15 | 14.86 | 17.30 | 13.96 |
| 1.5 | 16.87 | 20.13 | 15.69 | 19.88 | 15.60 |
| 2.0 | 18.48 | 23.07 | 17.74 | 22.79 | 17.12 |
| 2.5 | 21.16 | 25.22 | 20.15 | 24.38 | 19.55 |
| S.Ed | 3.81 | 4.33 | 3.51 | 4.10 | 3.33 |
| CD (P = 0.05) | 7.63 | 8.76 | 7.05 | 8.20 | 6.50 |

spp. (24.38 mm), *Bacillus* spp. (21.16 mm) and *Pseudomonas* spp. (21.00 mm). Lowest zone of inhibition was recorded in *Escherichia* spp. (20.15 mm).

The effect of Lactic acid on the inhibition of growth of five fresh cut vegetables spoilage bacterial strains *viz., Bacillus* spp., *Listeria* spp., *Escherichia* spp., *Salmonella* spp., and *Pseudomonas* spp. was studied and the results were presented in Table – 2. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of lactic acid from 0.5 to 2.5 %. Highest zone of inhibition was recorded against *Listeria* spp. (22.68 mm), *Salmonella* spp. (23.17 mm), *Bacillus* spp. (19.81 mm) and *Escherichia* spp. (17.63 mm). Lowest zone of inhibition was recorded in *Pseudomonas* spp. (16.28 mm).

Table-2. Effect of Lactic acid on the inhibition of Fresh cut vegetable spoilage bacteria by Agar well diffusion method

| Lactic acid (Conc)% | *Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | *Bacillus* spp | *Listeria* spp | *Escherichia* spp | *Salmonella* spp | *Pseudomonas* pp |
| Control | NZ | NZ | NZ | NZ | NZ |
| 0.5 | 12.20 | 15.56 | 10.95 | 13.70 | 10.65 |
| 1.0 | 13.09 | 16.35 | 11.82 | 15.26 | 11.29 |
| 1.5 | 15.25 | 18.92 | 13.60 | 16.20 | 12.41 |
| 2.0 | 17.38 | 20.34 | 16.71 | 19.63 | 14.50 |
| 2.5 | 19.81 | 22.68 | 17.63 | 21.17 | 16.28 |
| S.Ed | 3.20 | 3.95 | 2.91 | 3.57 | 2.73 |
| CD (P = 0.05) | 6.40 | 7.90 | 5.95 | 7.15 | 5.70 |

NZ – No zone of inhibition

Table-3. Effect of Citric acid on the inhibition of Fresh cut vegetable spoilage bacteria by Agar well diffusion method

| Citric acid (Conc)% | *Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | *Bacillus* spp | *Listeria* spp | *Escherichia* spp | *Salmonella* spp | *Pseudomonas* spp |
| Control | NZ | NZ | NZ | NZ | NZ |
| 0.5 | 12.85 | 14.54 | 10.22 | 13.69 | 9.06 |
| 1.0 | 14.26 | 17.49 | 11.60 | 15.23 | 11.51 |
| 1.5 | 15.33 | 19.22 | 14.36 | 16.10 | 13.89 |
| 2.0 | 17.57 | 21.10 | 16.81 | 19.28 | 16.20 |
| 2.5 | 19.20 | 24.76 | 18.93 | 21.34 | 17.12 |
| S.Ed | 3.36 | 4.11 | 3.13 | 3.70 | 2.63 |
| CD (P = 0.05) | 6.74 | 8.23 | 6.25 | 7.40 | 5.30 |

NZ – No zone of inhibition

The effect of citric acid on the inhibition of growth of five fresh cut vegetables spoilage bacterial strains *viz., Bacillus* spp., *Listeria* spp., *Escherchia* spp., *Salmonella* spp., and *Pseudomonas* spp. was studied and the results were presented in Table–3. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of malic acid from 0.5 to 2.5 %. Highest zone of inhibition was recorded against *Listeria* spp. (24.76 mm), *Salmonella* spp. (21.34 mm), *Bacillus* spp. (19.20 mm) and *Escherichia* spp. (18.93 mm). Lowest zone of inhibition was recorded in *Pseudomonas* spp. (17.12 mm).

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