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Microbial Spoilage of Modified Atmosphere Packaging on Fruits and Vegetables

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Abstract: A study was conducted to investigate the influence of microbiological activity on different quality factors of fruit and vegetable during storage. The fresh produce samples were collected from modified atmosphere packaging shed and assayed by enumerative tests for total aerobic bacteria, total coliforms and *Escherichia coli*. These samples were also analyzed for *Salmonella*, *Pseudomonas fluorescence* and *Erwinia carotovora*. For strawberry, geometric mean indicator levels ranged from bacterial count 2.6 to 10.7 log cfu/g less than fungal count ranged from 0.6 to 3.9 log cfu/g. For spinach leaves, geometric mean indicator levels ranged from bacterial count 3.0 to 11.2 log cfu/g less than fungal count ranged from 1.5 to 4.2 log cfu/g. Sodium benzoate and benzoic acid were capable of suppressing the growth of major spoilage microorganisms of intermediate fruit and vegetables. However, a number of studies have demonstrated that compounds existing in many chemical preservatives also possess antimicrobial activity. The most effective chemical preservatives were tested for their efficacy of inhibiting microbial growth in inoculated fresh fruit and vegetables.

Key words: Microbial Spoilage · Bacteria · Fruits · Vegetables And Chemical Preservatives

INTRODUCTION

Fruits and vegetables have been associated with outbreaks of food borne disease in many countries. Organisms involved include bacteria, viruses and parasites. These outbreaks vary in size from a few persons affected to many thousands. Contamination of vegetables may take place at all stages during pre and post-harvest technique [1]. Unsafe water used for rinsing the vegetables and sprinkling to keep them fresh is also a source of contamination [2]. Other possible sources of microorganisms include soil, faeces (Human and animal origin), water (Irrigation, cleaning), ice, animals (Including insects and birds), handling of the product, harvesting and processing equipment and transport [3].

Fresh fruits have an external toughness, water proof, wax-coated protective covering, or skin that functions as barrier for entry of most plant pathogenic microbes. These microbes get associated with fruits, since a variety of sources such as the blowing air, composited soil, insects as *Drosophila melanogaster* or the fruit fly inoculate the skin/outer surface with a variety of Gram-negative bacteria (Predominantly *Pseudomonas*, *Erwinia* and *Lactobacillus*) [4]. Likewise, hand-picking the fresh produce inoculates the fruit surfaces with *Staphylococcus*. These microbes are restrained to remain outside on fruit surface as long as the skins are healthy and intact. Any cuts or bruises that appear during the post harvest processing operations and packaging allow their entry to the less protected internal soft tissue [5].

The survival or growth of contaminating microorganisms is affected by intrinsic, extrinsic and processing factors. Factors of importance are nutrient composition, pH, presence of scales and fibers, redox potential, temperature and gaseous atmosphere. Mechanical shredding, cutting and slicing of the produce open the plant surfaces to microbial attack [6]. Fruits contain high levels of sugars and other nutrients and they possess ideal water activity for microbial growth, their low pH makes them particularly susceptible to fungal spoilage because a big part of the bacterial competition is eliminated since most bacteria refer near neutral pH. Surveys of raw fruits demonstrate that there is potential for a wide range of these products to become

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contaminated with microorganisms, including human pathogens. Outbreaks of gastrointestinal illness have been reported for intact products or minimally processed products, sprouted seeds and unpasteurized fruits [7]. The present study is focused on the isolation of spoilage microorganisms form vegetables and fruits and its control by using chemical preservatives.

MATERIALS AND METHODS

Sample Collection: Fruit (Strawberry) and vegetable (Spinach leaves) under modified atmosphere packaging samples used in the present study were purchased from the leading market on the four different places in Tamil Nadu, India.

Isolation and Identification of Bacteria from the Samples: One gram of spoiled strawberry and spinach leaves samples were aseptically transferred into 100 ml of sterile distilled water and shaked it well, the dilutions were made into 10^{-1}. From this dilution, again 1 ml sample was transferred into 10 ml of sterile distilled water and the dilutions were made into 10^{-2}. Likewise, the samples were serially diluted up to 10^{-6}. Pour plate technique was used for the isolation of bacteria in spoiled fruit and vegetable sample. Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar slants and stored at 4°C. The bacterial isolates were identified by staining techniques, motility test, platting on selective medium and biochemical tests.

Determination of Antimicrobial Activity of Bacterial Isolates: Agar well diffusion method was followed for evolution of antibacterial activity of five bacterial samples isolated from strawberry and spinach leaves. Sterilized nutrient agar medium was prepared and poured in sterile petriplates and allowed for solidification. The stainless steel cork borer used to make the one cm diameter well. Different concentrations chemical preservatives like 200 µl, 400 µl, 600 µl, 800 µl and 1000 µl were added in wells and incubated at 37°C for 24 hrs, after which they were observed for the zone of inhibition. Diameter of inhibition zones was calculated and expressed in mm.

RESULTS AND DISCUSSION

Six different bacterial isolates including *Salmonella* sp., *E. coli* and *Pseudomonas* sp. were isolated in the present research from strawberry and spinach leaves. The five chemical preservatives showed the zone of inhibition against the five spoilage and pathogenic microorganisms which was tested by Well diffusion method. The effect of Benzoic acid on the inhibition of growth of bacteria isolated from spoiled strawberry and spinach leaves samples were determined. Benzoic acid exhibited maximum zone of inhibition against *Salmonella enterica* (32 mm) followed by *Escherichia coli* (30 mm), *Erwinia carotovora* (25 mm) and *Staphylococcus aureus* (21 mm). The least zone of inhibition was observed in *Bacillus cereus* (19 mm) and *Pseudomonas fluorescence* (18 mm).

Sodium benzoate exhibited maximum zone of inhibition was observed against *Pseudomonas fluorescence* (36 mm) followed by *Staphylococcus aureus* (32 mm) and *Escherichia coli* (25 mm). The least zone of inhibition was observed in *Salmonella enterica* (24 mm), *Bacillus cereus* (23 mm) and *Erwinia carotovora* (22 mm). Among the five chemical preservatives showed higher inhibitory activity benzoic acid against bacteria *Salmonella enterica* (32 mm), *Escherichia coli* (30 mm) and sodium benzoate against *Pseudomonas fluorescence* (36 mm) and *Staphylococcus aureus* (32 mm) (Table 1).

Of the 2 samples tested in this study, sodium benzoate and benzoic acid exhibited strong antibacterial activities. The total viable counts in this study were within the range reported that, the bacterial loads on the spoiled spinach leaves range was from hundreds upto millions per square centimetres ($10^2 – 10^7$ numbers/cm^2) of leaves [8, 9]. Vegetables may be contaminated with pathogenic microorganisms during growing in the field or during harvesting, post harvesting, handling, processing and distribution. Therefore, vegetables may act as a reservoir for many microorganisms from which they will be colonized inside these fruit and vegetable and infect susceptible host. Almost any ready to eat vegetables that have been contaminated with pathogens either from the environment or from human or animal faeces or through storage, processing and handling could potentially cause disease [10]. A wide variety of acids, their salts and derivatives are used as chemical preservatives. Acids can be added to foods to lower the pH value which can eliminate the growth of certain microbial populations [11]. It is evident that using 0.05% of sodium benzoate or sodium sorbate is an option which the processors have in order to increase substantially the safety of fruit and vegetable [12, 13]. Chemical preservatives used in India culinary have been reported to exhibit suppressing action on many foods borne pathogens [14].

Table 1: Antibacterial activity of benzoic acid on strawberry and spinach leaves spoilage bacteria using agar well diffusion method

	Diameter of inhibition zone (In mm)					
Benzoic acid (μg ml^{-1})	*Salmonella enterica*	*Erwinia carotovora*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*
0	NZ	NZ	NZ	NZ	NZ	NZ
200	14	12	09	13	10	11
400	20	16	14	15	12	13
600	25	18	12	23	14	15
800	30	20	16	27	17	19
1000	32	25	19	30	19	21

NZ - No zone of inhibition

Table 2: Antibacterial activity of sodium nitrite on strawberry and spinach leaves spoilage bacteria using agar well diffusion method

	Diameter of inhibition zone (In mm)					
Sodium nitrite (μg ml^{-1})	*Salmonella enterica*	*Erwinia carotovora*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*
0	NZ	NZ	NZ	NZ	NZ	NZ
200	NZ	NZ	07	10	11	NZ
400	12	13	09	15	14	11
600	13	11	12	17	16	13
800	20	17	14	22	18	16
1000	24	22	18	25	21	18

NZ - No zone of inhibition

Table 3: Antibacterial activity of potassium nitrite on strawberry and spinach leaves spoilage bacteria using agar well diffusion method

	Diameter of inhibition zone (In mm)					
Potassium nitrite (μg ml^{-1})	*Salmonella enterica*	*Erwinia carotovora*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*
0	NZ	NZ	NZ	NZ	NZ	NZ
200	NZ	10	NZ	NZ	NZ	NZ
400	10	14	09	12	11	NZ
600	15	17	11	16	13	10
800	19	22	15	21	17	17
1000	20	25	18	21	23	19

NZ - No zone of inhibition

Table 4: Antibacterial activity of sodium benzoate on strawberry and spinach leaves spoilage bacteria using agar well diffusion method

	Diameter of inhibition zone (In mm)					
Sodium Benzoate (μg ml^{-1})	*Salmonella enterica*	*Erwinia carotovora*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*
0	NZ	NZ	NZ	NZ	NZ	NZ
200	11	13	14	16	26	2 0
400	13	14	11	12	28	25
600	15	16	13	17	30	29
800	20	19	15	22	33	30
1000	24	22	23	25	36	32

NZ - No zone of inhibition

Table 5: Antibacterial activity of potassium sorbate on strawberry and spinach leaves spoilage bacteria using agar well diffusion method

	Diameter of inhibition zone (In mm)					
Potassium sorbate (μg ml^{-1})	*Salmonella enterica*	*Erwinia carotovora*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*
0	NZ	NZ	NZ	NZ	NZ	NZ
200	NZ	NZ	NZ	NZ	12	14
400	NZ	11	NZ	10	14	16
600	13	13	11	12	17	18
800	15	15	13	14	19	21
1000	17	21	15	20	23	25

NZ – No zone of inhibition

CONCLUSIONS

This present study showed that the product preserved with chemical preservatives retain maximum overall acceptability during storage capable of inhibiting pathogenic and spoilage microorganisms. These chemical preservatives are widely used in the food industry and are generally regarded as safe (GRAS). Hence, they may be considered as safety and niche preservatives acceptable by the muscle food industry.

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