OPEN ACCESS Freely available online

Journal of
Microbial & Biochemical Technology

Research Article

Laboratory Based Experimental Study on Microbial Spoilage of Commercially Available Fruits

Sarmad Muhammad Soomar* and Salman Ranani

Department of Psychiatry, Aga Khan University Hospital, Karachi, Pakistan

ABSTRACT

Microorganisms are everywhere. They can be found in the air, in water, in soil, on animals and even on humans. Some are beneficial, such as those used to make fermented dairy and meat products. Others cause spoilage of various food products. Eating fruits is a healthy practice due to its nutritional composition but when it gets spoiled by microbes, it can be harmful for human consumption. Microorganisms have been reported to cause extensive deterioration of fruits. Some of these microorganisms cause rotting, discoloration or fermentation of the fruits which affect their preservation. The study was done to identify and analyse microbial diversity that causes the spoilage. Pour plate method was used for the isolation of microbes from spoil fruit. A portion of the fruit was aseptically inoculated into the beaker; it was homogenized and then diluted. The colonies were identified by standard bacteriological procedures. Gram's staining was performed to determine if the organism is gram negative or gram positive. Further confirmatory biochemical tests were done such as catalase, coagulase and oxidase. The identification of the isolated fungi was done both macroscopically and microscopically. This study and experiments revealed that the rotten or spoiled fruit possess appreciable number of microbes. The Microorganisms isolated and observed were bacteria and fungi majorly. This is due to various processes taking place in the rotten fruit which favoured bacterial and fungal growth. It could also be as a result of the moisture content of the fruit as well as the difference in the nutritional composition of the fruits. This work finds that there are microorganisms that could be responsible for inducing spoilage in the fresh fruit. Fruits are a good source of nutrient and could be used for many applications. However to reduce the susceptibility of the fruit to microbial spoilage and to ensure its effectiveness in different applications and safety measures should be taken. Hence if you want to prevent spoilage of food by micro-organisms, you must remove the conditions which are appropriate for their growth and preserve them with the best possible techniques.

Keywords: Microbial; Spoilage; Fruits; Food safety

INTRODUCTION

Fruits occupy a very important role in human diet by supplying the essential development and growth factors such as vitamins and essential minerals in human every day diet and that can assist to keep a good and normal health. One of the warning factors that manipulate the fruits economic worth is the quite short shelf-life period caused by pathogens attacked. It is expected that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries. If the intrinsic and extrinsic conditions are appropriate, microorganisms grow rapidly and make attractive and appealing food into a sour, foul-smelling or fungus-covered mass suitable only for the garbage can [1-4]. Microbial growth in foods can lead to visible changes, including a variety of colours caused by spoilage organisms. An alteration in the appearance, smell or flavour of fruit that makes it objectionable to the user is called fruit spoilage. Fruits are easily spoiled and susceptible to infection by bacteria fungi and viruses. It is estimated one-fourth of the harvested fruits are spoiled before consumption. Microbial spoilage in fruits varies not only with the kind, variety and composition of fruit. The character of the spoilage depends upon the product attacked and the attacking organism. Spoilage of fresh fruits usually occurs during storage and transport. Osmophilic micro flora spoil fruits and vegetables mainly during propagation of their natural acid tolerant and fresh fruits are more liable to spoilage for the reasons that liquid (watery) contents are in contact with air and microorganisms from surroundings. Microbial invasion of fruits can occur during various stages of its development. Another factor that contributes to spoilage is the environmental exposure for certain time. Each year 20% of all fruits produced are lost due to spoilage. Majority of microorganisms are soil inhabitants that are spoilage factors of fruits and are responsible for maintaining an

Correspondence to: Sarmad Muhammad Soomar, Department of Psychiatry, Aga Khan University, Karachi, Pakistan, Tel: +923322383509; E-mail: soomarsarmad@gmail.com

Received: June 15, 2019, **Accepted:** August 27, 2019, **Published:** September 03, 2019

Citation: Soomar SM, Ranani S (2019) Laboratory Based Experimental Study on Microbial Spoilage of Commercially Available Fruits. J Microb Biochem Technol. 11:4. doi: 10.35248/1948-5948.19.11.423

ecological balance. They are transmitted by soil particles, airborne spores and irrigation water [5-12].

The fruit spoilage occurs seasonally, typically in the spring or summer, and occurs most commonly in apple and orange. In this regard all microorganism has a most favourable (optimal) temperature for growth. Thus avoiding storage at this temperature or near this temperature reduces the rate of spoilage. Fruit Spoilage can be caused by even smaller subset of Bacteria and fungi. Some ideal conditions favour the growth and survival of many types of microorganisms that spoil fruits. Damage (External damage such as bruising, cracks, and punctures) or compromised fruits more likely to develop colonization and lesion thus establish the growth of spoilage microbes. Microbial contamination or spoilage depends upon storage time and storage conditions as well. For instances Apples are stored in large rooms and controlled atmosphere. Clean and new (fresh) fruits produced and distributed commercially go through these events of maturity and during that a number of physiological and compositional alterations take place [13-17].

Bacteria are found in reduced numbers on fresh fruits because of decreased pH. Colonization of bacteria with the fruits external surfaces includes both the gram positive as well as gram negative bacteria indicating diverse morphological structures such as bacillary rods to spherical coccid cells [18,19].

More utilization of fruits has straight pressure on economy in positive manner but it can become negative also when food borne disease occur. Food borne pathogens pollute fruit surfaces and contaminated with feces and may impure washing water and allow internalization aids their existence under the presence of acidic conditions of fruits thus some strains of Escherichia coli, Shigella, and Salmonella may continue to exist for long time (days to weeks) in acidic environment by adapting their internal pH. Unpasteurized fruit juices commonly available at street (very usual in Asia) are encountered with Salmonella and Staphylococcus aureus [20,21].

Fungal contamination in fruits is reported most widespread micro flora and also their most important pathogens, without neglecting the reality that bacterial spoilage is also an important cause of the fruit spoilage. Thus, the rate of bacteria and fungi as the prevalent organisms in fruits is of crucial importance in this learning as a little of these micro flora have the possibility to generate toxins which are secondary metabolites of organisms that are poisonous/toxic and/or carcinogenic in some situations impacting badly [22].

MATERIALS AND METHODS

Three types of fruits, Apple, Banana, and Grape were purchased from market in their individual packages as three sets of each fruit. The fruits were collected in a polythene bag and transferred into the laboratory. One set of these three fruits were exposed for twenty four hours, second one for forty eight hours and third one for seventy two hours, in order to isolate the fruit spoilage microbes at different exposure time period to know the degree of fruit spoilage by microorganisms at standard temperature using safety techniques at laboratory.

Twenty seven petri plates, three conical flasks for preparing three different types of media, three small beakers for homogenizing fruit, one large beaker for distilled water, around fifty test tubes for preparing dilution with tenth fold dilution method, pipettes, gram staining kit and other biochemical reagents, slides, microscope and oil immersion were managed for the experiment. Media used for the work were Nutrient Agar, Mannitol Salt Agar, Sabraoud Dextrose Agar, MacConkey Agar and Brain Heart Infusion Broth. The media were prepared using manufacturer's instruction and sterilized using autoclave at 121 for fifteen minutes.

Pour plate method

Pour plate method was used for the isolation of microbes from spoil fruit, In a pour plate, a small amount of inoculum from a broth culture is added by pipette to the centre of a Petri dish. Cooled, but still molten, agar medium in a test tube or bottle is then poured into the Petri dish. The dish is then rotated gently, or moved back and forth, to ensure that the culture and medium are thoroughly mixed and the medium covers the plate evenly. A portion of the fruit was aseptically inoculated into the beaker and homogenized than diluted through tenth fold dilution method and poured on to the prepared media for the isolation of microbes.

Homogenizing method

The methods used to detect and isolate spoilage microorganisms are mainly based on cultural procedures. For example, extracted microorganisms from the fruits (1:10 dilution) using sterile 0.1% peptone water and 0.5% sodium chloride, softened this preparation by stomaching for 1 min and using the following methods for numbering different microorganisms: Nutrient agar (NA) with incubation at 37°C for 1 or 2 days for aerobic plate counts; Manitol Salt Agar with incubation at optimum temperature for 1 or 2 days for yeasts and molds; Sabraoud Dextrose Agar (SDA) agar with optimum temperature for 1 or 2 days or it can take more (around a week).

In this case the objective of sample preparation is to isolate as many of the possible microorganisms as probable from the sample surface or deep from rust or lesion for subsequent isolation and detection. Several different strategies may be used to release microorganisms, and all typically begin by adding the sample to a volume of sterile diluent to obtain a 1:10 dilution in a sterile water, distil water can be used for this purpose. Phosphate-buffered saline, Butterfield's buffer, and 1% buffered peptone water are all acceptable diluents for this purpose and can be prepared easily in the laboratory or purchased reformulated. Isolating the microbes can be accomplished by palpating the sample in a homogenizer (automatic or manual) for up to 2 min, or by grinding the sample in a sterile, commercial blender for up to 60s, or by vigorous shaking on a wrist-action shaker for few minutes. Indeed, when sample preparation must be conducted outside the laboratory setting, freeing surface microbes can be accomplished, although less efficiently and with lower yields than the aforementioned methods, by hand shaking the sample bag for up to 2 min.

The homogenizer method, probably the most widely applied in the food industry, is rapid, does not come into physical contact with the diluted sample (does not require re-sterilization between samples) and reportedly provides a high rate of recovery of viable microbes from the sample. Blending the sample is rapid and efficient but the blender jar and blades must be re-sterilized between samples (or multiple blender jars must be used). Shaking the diluted sample on a wrist-action shaker is efficient and depending upon the length of the shaker arms up to 16 samples can be prepared simultaneously and the shaker reused immediately as the sample does not come

into direct contact with the equipment. Another advantage of agitation by a wrist-action shaker is that the sample remains more or less intact. This is unlike either the homogenizer or blender that macerates the tissue and makes subsequent sample handling steps such as pipetting more difficult. A relatively new piece of equipment the Pulsifier offers the same advantage as the wrist-action shaker by preparing the sample with very little maceration of the sample. The compared total viable microbial growth and total coliform recovered from samples of 30 different fresh vegetables, with the homogenizer and with the Pulsifier and found no difference in viable recovery between the two methods. Irrespective of the initial sample preparation step, the next step depends on whether the investigator is interested in attempting quantitative recovery of a specific pathogen (or pathogens) or simply desires to determine if the microbe of interest is present on the sample. Quantitative recovery can be difficult if a suitably selective medium for the pathogen of interest does not exist. In this instance, it is typically necessary to spread the dilution on the plate directly onto prepared several different media and subsequently identify those colonies similar to the microbe of importance.

However, if a suitable selective medium does exist, the serial dilution is followed by spread-plating (0.1 ml) and incubation. Incubation time and temperature depend very much on the temperature range of the microbe of interest, optimum temperature is best, compared with the typical temperature range of the background flora the investigator wishes to suppress. Many spoilage microbes develop very distinctive lesions depending upon the fruit. For this reason, initial diagnosis often is conducted in the field or in the packing facility based on macroscopic appearance of the lesion [23].

After homogenising the spoiled fruit sample was serial diluted in distil water. Each type of fruit (with different exposure time) was used in performing this. Serial dilutions are made by making the same dilution step over and over using the previous dilution as the input to the next dilution in each step. Since the dilution fold is the same in each step. The technique used to make a single dilution is repeated sequentially using more and more dilute solutions as the "stock" solution. At each step, 1ml of the previous dilution is added to 9ml of solvent. Each step results in a further 10 fold change in the concentration from the previous concentration. The values shown in the tubes are the amount (in ml) of the stock solution present in each ml of the dilute solution. When the dilution is prepared pour it on the prepared media plates. Inoculate labelled empty petri dish with specified mL diluted specimen. Pour 15 mL of melted, 45°C prepared media (Nutrient Agar, Mannitol Salt Agar, Sabraoud Dextrose Agar) into the inoculated petri dish. Mix thoroughly by tilting and swirling the dish. Do not slop the media over the edge of the petri dish. Allow the media to completely gel without disturbing it. (About 10 minutes). Invert and incubate at 37°C for 24-48 hours. After incubation, observed colonies was counted and then isolated. The bacterial isolates after obtaining pure culture was further subjected to gram staining reactions to aid in identification after which they were examined for carbohydrate production of indole from tryptophan, citrate utilization, catalase production coagulase and oxidase test. The bacterial isolates were also identified by comparing their characteristics with those of known taxonomy [24].

The colonies were identified by standard bacteriological procedures. Gram's staining was performed to determine if the organism is gram negative or gram positive. A smear of the test organism was made on a clean slide, dried and covered with crystal (Figure1).

Analysis of the samples

The colonies were identified by standard bacteriological procedures. Gram's staining was performed to determine if the organism is

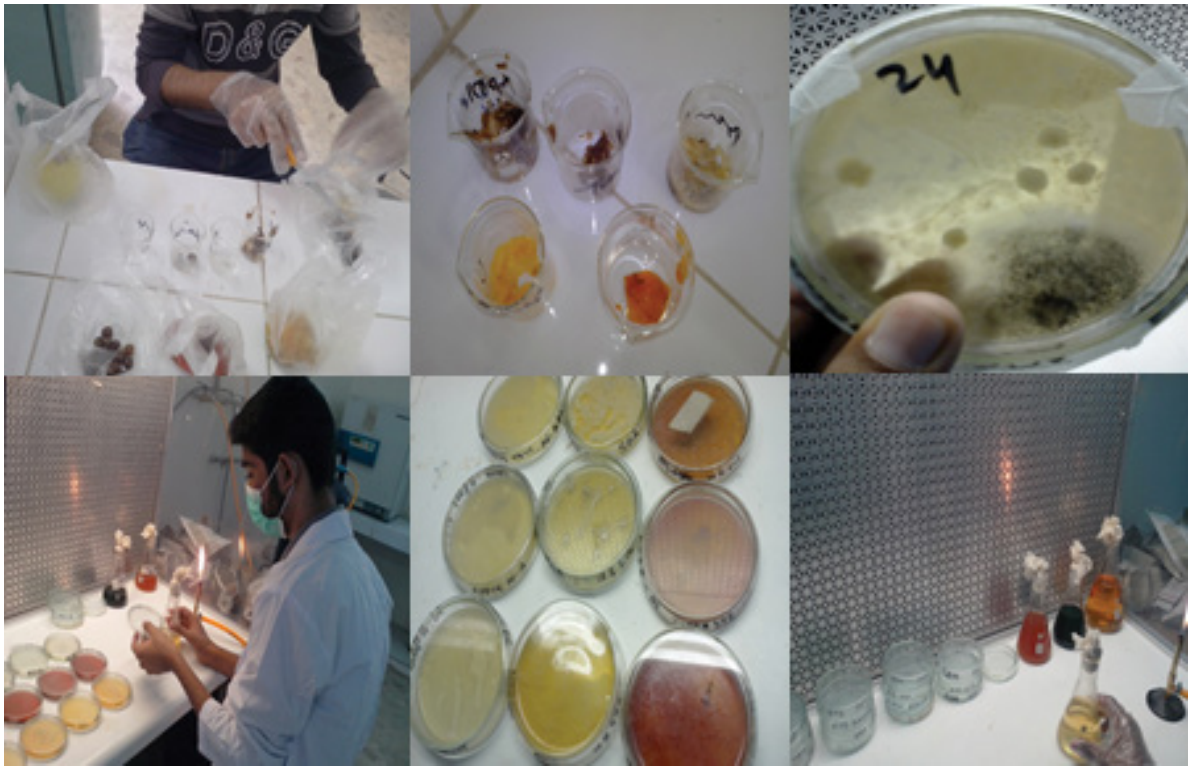

Figure 1: Collage of pictures from the experiment shows how it describes the process of whole experiment. Beginning from the segregation of fruits, preparation of petri dishes and other mentioned laboratory tests.

gram negative or gram positive. A smear of the test organism was made on a clean slide, dried and covered with crystal violet for 30 to 60 s. It was washed off with clean water and was covered with iodine for 30 to 60 s and later washed off with clean water. The slide was decolorized with acetone-alcohol, and was washed immediately with clean water and was covered again with neutral red stain for 2min, and was washed off with clean water. The back of the slide was wiped clean and placed in a draining rack for the smear to air dry. The slide was examined microscopically with the oil immersion lens after the application of the oil on the slide. Gram-positive bacteria gave a dark purple colour while Gram-negatives give a red colour. Series of biochemical tests such as catalase, citrate, coagulase, oxidase, and urease were performed on the bacteria isolates in accordance with procedures to analyses the bacterial growth. The identification of the isolated fungi was done both macroscopically and microscopically. The gross morphology of the fungal growth on plates was studied including their colours and the actively growing mold using morphological characters such as the absence or presence rhizoid, colour, and micro-morphology of their sporulation structures and conidia.

When microbial analysis is done culture is preserved or stored by making pure culture, Sterilize your loop until the entire thin length of the loop, not just the end, is red hot in the burner flame. Allow the loop to cool for a few seconds. Pick up the plate from which you want to transfer the microbial growth. Put your sterile loop into the mix culture plate to obtain a microbial sample. Pick up the tube of Brain Heart infusion broth into which you want to transfer (inoculate) the microbial. Repeat the procedure for all plates [25].

RESULTS AND DISCUSSION

Enumeration, Isolation and Identification of Microorganisms associated with spoilage of Fruits (Apple, Banana and Grapes) were studied using standard microbiological methods. Apple turned brown after 24 hours of exposure, black after 48 hours and a large dark black path with unpleasant smell formed after 72 hours. Grapes turned slightly brown or yellow brown after 24 hours, complete brown after 48 hours and black after 72 hours. Banana turned brown, black and completely spoiled with rotten smell during different exposure timings (Table 1).

Organisms isolated and identifies from apple after 24 hours were *Staphylococcus spp*., *Escherichia coli*, *Klebsiella spp*., *Salmonella spp*., *Pseudomonas spp*., *Vibrio spp*. and *Listeria spp*. Other than these species *Clostridium pasteurianum BB*, *Bacillus acido caldarius* were isolated and isolated from apple after 48 hours. Penicilium expansum soft rot of apples and *Aspergillus Niger* were isolated also after 72 hours of exposure; it was a black and moldy which was unpleasant. Organisms isolated from spoiled grapes after 24 hours were *Bacillus spp*. and *Staphylococcus spp*. The bacteria of *Cryptococcus spp*., *Gluconobacter spp*., *Acetobacter spp* and *Enterococcus spp*. were also isolated from grapes after 48 hours. *Candida spp* and *Aspergillus spp. (A. Niger)* were isolated after 72 hours. No any spoilage microbes were isolated from after 24 and 48 hours in Bananas except *Fusarium spp.* (fungi) that was systemic vascular infection found after 72 hours (Table 2).

The morphological characteristics of these microorganisms are diverse. *Staphylococcus* **is** a genus of Gram-positive bacteria. Under the microscope, they appear round, and form in grape-like clusters.

Table 1: Structure/texture of infected fruits.

Fruit	Exposure Time			
	24	48	72	
Apple	Brown	Black	Black large patch with un pleasant smell	
Grapes	Yellow	Brown	Brown	Black
Banana	Brown	Black Patch	Black with rotten smell	

Table 2: Microorganisms isolated.

Fruit	Exposure Time		
	24	48	72
Apple	*Staphylococcus* spp.	*Clostridium pasteurianum* BB	*Penicilium expansum*
	Escherichia coli	*Bacillus acidocaldarius*	*Aspergillus Niger*
	Klebsiella spp	-	-
	Salmonella spp.	-	-
	Pseudomonas spp.	-	-
	Vibrio spp.	-	-
	Listeria spp.	-	-
Grapes	*Bacillus* spp.	*Cryptococcus* spp.	*Candida* spp.
	Staphylococcus spp.	*Gluconobacter* spp.	*Aspergillus niger*
	-	*Acetobacter* spp.	-
	-	*Enterococcus* spp.	-
	-	-	-
Banana	-	-	*Fusarium* spp.

Escherichia coli are a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus. *Klebsiella spp* is a genus of non-motile, Gram-negative, oxidase- negative, rod-shaped bacteria with a prominent polysaccharide-based capsule**.** *Salmonella* is a genus of rod-shaped, Gram- negative bacteria. *Pseudomonas* is a genus of Gram-negative, aerobic gamma proteo bacteria. Vibrio is a genus of Gram- negative bacteria possessing a curved-rod shape, several species of which can cause foodborne infection**.** *Listeria* species are facultative anaerobic, non-spore forming bacilli. Clo*stridium pasteurianum* BB is a saccharolytic and spore-forming obligate anaerobe bacteria. *Cryptococcus spp* is a genus of fungus. These fungi grow in culture as yeasts. *Gluconobacter spp* & *Acetobacter spp* belongs to family Acetobacteraceae and they are Gram-negative, aerobic, rod-shaped bacteria. *Enterococcus* is a genus of lactic acid bacteria of the phylum Firmicutes. *Enterococci* are Gram-positive cocci that often occur in pairs (diplococci) or short chains. Candida spp is a genus of yeasts; *Candida* appears as large, round, white or cream colonies. *Aspergillus* is a fungal genus, colonies are usually fast growing, white, yellow-brown, brown to black or shades of green, and they mostly consist of a dense felt of erect conidiophores.

On exposure fruits turned to brown, black and rotten. For example in apple this is caused by an enzyme (polyphenol oxidase and catechol oxidase are two common examples) that reacts with oxygen in the air and an iron or copper cofactor in the fruit. A cofactor is a component that is necessary for a certain enzymatic reactions to happen. The fruit starts to oxidize, when electrons are lost to another molecule (in this case the air), and the food turns brown. In other words, it's like edible rust on your food. Oxidation can be prevented or slowed down by not allowing oxygen to get to the surface of the fruit. To accomplish this, you can cook the food,

which destroys the enzyme. It is also possible to prevent browning without cooking by covering the fruit (preventing air from reaching the fruit), or by lowering the pH on the surface, making it more acidic.

Our results generally demonstrated high microbial diversity across the three fruits we analysed. Diverse phyla were well represented by the sequences in at least one produce type. The microbial taxa we observed were consistent with findings from other studies that have used culture-independent techniques to describe taxon abundances. We found the microbial communities in spoiled Apple, Banana and Grapes to be numerically dominated by bacteria and fungi. We observed distinct bacterial and fungal communities and substantial variation in bacterial richness across the produce types we analysed. There are different spoilage bacteria which grow well at room temperature. Bacteria can cause fruits to get mushy or slimy, or meat to develop a bad odour.

RECOMMENDATIONS AND CONCLUSION

The demand for fruits has been increasing due to their health benefits. Due to change in dietary, social habits and preservation methods have led to increase in disease outbreaks linked mainly to fresh fruit in recent years. Fruits are very important and have high dietary and nutritional qualities. Microorganisms are naturally present on all food stuff and can also be brought in by outside elements (wind, soil, water, insects, animals, human handling). They can become contaminated during growing, harvesting and transport of the raw materials, and/or processing into edible products.

The prevalence of some fungi and bacteria demand that appropriate control measures should be employed if farmers expect a beneficial outcome of their product. Adequate Microbiological knowledge and handling practices of these products would therefore help to minimize wastes due to deterioration of fruits. Proper measures should be adopted while handling fruits to limit the level of microbial contamination. It is therefore important to prevent contamination and also try to create an environment that will not encourage the growth and multiplication of microorganisms. This study has shown that microorganisms causing diseases are present in the decaying fruits and this is of public health importance. However, still continuous experimental researches are recommended which are laboratory based and identify further aspects of fruit spoilage and food safety.

ACKNOWLEDGMENT

The authors themselves were involved in experimentation in the laboratory want to acknowledge efforts and support of the lab personals providing space for experiment and support in arrangement of equipment. Colleagues who supported us as team in practical experiments are also greatly acknowledged for their exemplary assistance and coordination. Since it was an academic project and experiment during the microbiology course, we also acknowledge faculty for guidance and support to carry out this project. The project was academic activity therefore there were no separate funding allocated for it and the basic and primary resources were arranged by authors themselves.

References

1. Dorby S, Zhu SJ. Improving quality and safety of fresh fruit and vegetables pro and post-harvest using biochemical agents and materials. Acta Horticulture. 2006;709:45- 51.
2. Beck R. A Chronology of Microbiology in Historical Context, ASM Press, Washington D.C. 2000.
3. Brock TD. Milestone in Microbiology: 1546 to 1940, ASM Press, Washington, D.C. 1999.
4. Riley LW, Remis RS, Helgerson SD, Cohen ML. Hemorrhagic colitis associated with a rare Escherichia coli serotype. N Engl J Med. 1983;308:681- 685.
5. Aneja KR, Jain P, Aneja R. A Textbook of Basic and Applied Microbiology, 1st edn, New Age International Publishers, New Delhi, India, 2008.
6. Bevilacqua A, Corbo MR, Sinigaglia M. Use of natural antimicrobials and high pressure homogenization to control the growth of Saccharomyces bayanus in apple juice. Food Control. 2012;24:109-115.
7. Pelczar, MJ, Chan ES, Krieg NR. Microbiology, 5th Ed. Tata McGraw-Hill Publishing Company Limited, New Delhi. 2005; pp 619.
8. Janisiewicz WJ, Tworkoski TJ, Sharer C. Characterizing the mechanism of biological control of postharvest diseases on fruits with a simple method to study competition for nutrients. Phytopathology. 2000; 90:1196-1200.
9. Walls I, Chuyate R. Alcylobacillus historical perspective and preliminary characterization study. Dairy. Food Environ Sanit. 1998;18(8):499- 503.
10. Miedes E, Lorences EP. Fruit cell wall degradation. J Agric Food Chem. 2004;52(26):7957-63.
11. Watkins CB, Miller WB. A summary of physiological processes or disorders in fruits. 2004
12. Watada AE, Norris KH, Worthington JT, Massie DR. Estimation of Chlorophyll and Carotenoid contents of whole fruit by light absorbance technique. J Food Sci. 1984;41:329-332
13. Lindow SE, Desurmont C, Elkins R, McGourty G, Clark E, Brandl MT. Occurrence of Indole-3-Acetic Acid–Producing Bacteria on Pear Trees and Their Association with Fruit Russet. Phytopathology. 1998;88(111):149-1157.
14. Raybaudi-Massilia RM, Mosqueda MJ, Soliva FR, Mart´ın-Belloso O. Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. Comprehensive Reviews in Food Sci Food Safety. 2008;8(3):157-180.
15. Lima Tribst AA, De Souza SA, De Massaguer PR. Review: microbiological quality and safety of fruit juices past, present and future perspectives Microbiology of fruit juices Tribst et al. Critical Rev Microbiol. 2009;35(4):310-339.
16. Tournas VH, Heeres J, Burgess L. Moulds and yeasts in fruit salads and fruit juices. Food Microbiol. 2006;23(7):684-688.
17. Vantarakis A, Affifi M, Kokkinos P, Tsibouxi M, Papapetropoulou M. Occurrence of microorganisms of public health and spoilage significance in fruit juices sold in retail markets in Greece. Anaerobe. 2011;17(6):288-291.
18. Tseng TC, Tzean S. Mycoflora and mycotoxins in dry bean (Phaseolus vulgaris) produced in Taiwan and in Ontario, Canada. Bot Bull Acad Sin. 1995;36:229-234.
19. Okigbo RN. Mycoflora and production of wine from fruit of sour sops. Int J Wine Res. 2009;1:1-9.
20. Osuide GE. Postharvest Losses Implication for Food Quality and Safety. A Paper Presented at NIFST 23rd National Conference/ Annual General Meeting Abuja, Nigeria.1999;19.
21. Mosqueda MJ, Raybaudi MRM, Mart BO. Non-thermal pasteurization

of fruit juices by combining high-intensity pulsed electric fields with natural antimicrobials. Innovative Food Science and Emerging Technologies. 2008;9(3):328-340.

22. Pitt JI, Hocking AD. The ecology of fungal food spoilage. In: Pitt JI, Hocking AD (eds), Fungi and Food Spoil. 2008; 5-8.

23. Oyeleke SB, Manga BS. Essentials of Laboratory Practical in Microbiology, 1st edn., Tobest Publishers. Minna Nigeria, 2008;28-62.

24. Downes FP, Ito K. Compendium for the microbiological examination of foods, 4th edn., Washington, DC: American Public Health Association. 2001.

25. Wongsaroj C. Isolation of gram-negative bacteria from cockroaches trapped from urban environment. Southeast Asian J Trop Med Public Health. 2004;35(3):681-684.