

## Microbial Evaluation of Branded Fruit Juices Sold in the City of Chakdara, Dir (Lower) Pakistan

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**Abstract:** The quality of fruit juices are not strictly maintained in developing and underdeveloped countries, the manufacturers are not concerned about the microbiological safety and hygiene of fruit juices because of lack of awareness and enforcement of the law. Contamination of the juices with bacteria leads to a number of diseases. Seven different samples of branded fruit juices were investigated to determine the involvement of bacteria in them. Nutrient agar plates were prepared and inoculated with selected juice samples. The plates were incubated for 24 hours at 37°C. After 24 hours, various colonies of microbes were produced at the surface of solid agar media in the plates and were identified by Gram staining and microscope. The pH of the juices was also determined. Various kinds of bacteria were detected in all the selected samples. The *Cocci* were observed in large quantity, *Bacilli* in moderate while *Spirilla* in minute quantity. The *Cocci* were mostly Gram Negative while the *Bacilli* were of Gram Negative type. The spirilla was absent in all the selected juice samples except mango. The pH of the strawberry juice was neutral while the pH of the rest of samples was on acidic side. The juice makers claim that these are properly sterilized and packed. However, the presence of these bacteria showed that the medium was not a properly sterilized one.

**Key words:** Fruit juice • Spirilla • Cocci • Bacilli • Sterilized

### INTRODUCTION

Fruit juices are becoming an important part of the modern diet in many communities of the world. They are nutritious and can play a significant role in a healthy diet because they offer good taste and many nutrients are founded naturally in fruits. Juices are available in their natural concentrations or in processed forms. Juice is prepared by mechanically pressing fresh fruits or is extracted by water. Fruit Juices have no fats and are cholesterol free, rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health maintenance. For example, orange juice is rich in vitamin C, an excellent source of bio-available antioxidant photochemical [1] and significantly improves blood lipid profiles in people affected by hyper-cholesterolemia [2]. Fruit juices promote detoxification in the human body [3].

In recent years the increasing consumer awareness has emphasized the requirement for microbiologically safe food. Since the Human food supply contains basically of plants and animals or small products derived from them, it is unfavorable that our food supply contains

microorganisms. When the micro-organisms involved are pathogenic (causes diseases), their association with our food is critical from a public health point of view. Serious health hazards due to presence of pathogenic microorganisms in food can lead to food poisoning outbreaks [4]. In Pakistan the chance of transmissions of disease through fruit juices are due to the unsatisfactory hygiene and adulteration practices. Micro-organisms are present both inside as well as outside of fruits and vegetables. At the time of consumptions the majority of bacterial population found on surface is usually Gram negative and belong to the Enterobacteriaceae. The inner tissues of fruits are usually regarded as sterile. However, some bacteria can be present in low number as a result of the uptake of water through certain irrigation or washing procedures [5].

Fruit juices are traditionally considered as susceptible to spoilage only by yeast, mycelial fungi and lactic acid bacteria [6]. Fruit juices with pH lower than four are considered as high in acid and are generally regarded as not being susceptible to spoilage by a variety of microorganisms [7]. The low pH is considered enough to

prevent the growth of almost all bacteria spore formers. Spores of *Clostridium botulinum* cannot germinate or produce the lethal botulinum toxin in an environment with a pH below 4.6 [6].

Pathogens can enter fruits through wounded surfaces that occur during growing or harvesting. But the main sources of contamination in fruit juices are untidy instruments and utensils, unhygienic water for dilution, dressing with contaminated ice, prolonged preservation without refrigeration, unhygienic environment often with swarming houseflies and fruit flies and airborne dust. Such juices have shown to be potential sources of bacterial pathogens notably *E. coli*, species of *Salmonella*, *Shigella* and *S. aureus* [8].

The quality of fruit juices is strictly maintained in developed countries under several laws and regulations but in many developing and underdeveloped countries, the manufacturers are neither concerned nor aware about the microbiological safety and hygiene of fruit juices because of lack of enforcement of the law. Thus the transmission of certain human diseases through juice and other drinks in recent years is a serious problem [9].

Primary objectives of this study were to assess the microbial quality of branded juices sold in Chakdara city. Standard culture techniques were used to determine the involvement of bacteria in the locally available branded fruit juices in the market.

#### MATERIALS AND METHODS

The branded fruit samples employed in this study were collected from local market. A total of seven different branded fruit juice samples were evaluated for the involvement of bacteria in them. Nutrient agar, petri dishes etc were sterilized in oven for 15 minutes at 121°C. Agar plates were prepared and inoculated with the juice sample through aluminum loop under laminar flow. The laminar flow was switched on about ten minutes before the conduction of inoculation process. The plates were taken up from laminar flow with care and were kept in incubator at 37°C. After 24 hours, various colonies of microbes were produced at the surface of solid agar media in the plates. These microbial colonies were located at different zones at the solid agar surface, some with large occupation and some with small. Various colonies were selected from the solid agar surface; minute quantities of these were taken through aluminum loop under laminar flow and spread over the slide. To identify the bacterial present in the juice samples the slides were subjected to Gram staining and microscopic examination.

pH of all juice samples were also determined by pH meter.

#### RESULTS

Various kinds of *Cocci* were present in the collected branded juices. The majority of them were gram positive while minor quantities were gram negative *Cocci* bacteria. The *Cocci* present were *monococcus*, *diplococcus*, *streptococcus* and *staphylococcus aureus*. The rod shaped bacteria were also detected in small quantity. The gram negative rods were detected in large quantity than gram positive rods. The vibrio and other gram negative bacilli were also detected in some samples. Spirilla were present in minute quantity. The results obtained are given in Table 1.

**pH of Selected Juice Samples:** The pH of all juice samples were determined through pH meter and are given in Table 2.

#### DISCUSSION

In this study 7 different branded fruit juice samples were investigated for their microbial quality. Fruit juices provide minerals, vitamins, proteins and carbohydrates [1-3]. Besides this they are carrier of a large number of microbes which can cause serious diseases [4, 5]. The bacteria which were observed are *cocci* in large quantity, *bacilli* in moderate amount while *spirilla* in minute quantity. Their details are given below.

In most of the samples the *Cocci* observed were Gram Positive while one Gram Negative *Coccus* observed in mango juice was of the *Neisseria* family. In rest of the sample no Gram Negative *Cocci* were observed. The result showed that these juices are resistant to growth of Gram Negative bacteria but are susceptible to Gram positive *Cocci* (Table 1).

Table 1 shows the *Bacilli* contamination in the selected samples. Here mostly Gram Negative *Bacilli* were observed in the samples. Gram positive *Bacilli* were present in strawberry, guava and mango juices. In rest of the samples Gram Positive *bacilli* were absent. Gram Negative *bacilli* were absent in strawberry and guava juices. It shows that these juices are susceptible to Gram Positive *bacilli* while resistant to Gram Negative. Except strawberry and guava juices all juices were susceptible to Gram Negative *bacilli*. *Vibrio* was observed in apple and pomegranate juices that are responsible of Cholera in human.

Table 1: Various types of bacteria present in selected juice samples.

S.No	Juice sample	Cocci		Bacilli		Spirilla	
		Gram positive	Gram Negative	Gram positive	Gram negative	Gram positive	Gram negative
1.	Strawberry	Monococcus, Diplococcus, Triplococcus, Tetracoccus, Staphylococcus aureus	Absent	Clostridia family	Absent	-	-
2.	Apple	Monococcus, Diplococcus, Triplococcus, Tetracoccus, Staphylococcus aureus	Absent	-	Vibrio	-	-
3.	Pomegranate	Monococcus, Diplococcus, Triplococcus, Tetracoccus, Staphylococcus aureus	Absent	-	Vibrio, Enterobacteriaceae family	-	-
4.	Guava	Monococcus, Diplococcus, Triplococcus, Tetracoccus, Staphylococcus aureus	Absent	Clostridia family	-	-	-
5.	Apple	Monococcus, diplococcus, Staphylococcus aureus, Tetracoccus	Absent	-	Enterobacteriaceae family	-	-
6.	Grapes	Monococcus, diplococcus, Streptococcus, Staphylococcus aureus	Absent	-	Enterobacteriaceae family, Yersinia	-	-
7.	Mango	Monococcus, Diplococcus, Triptococcus, Streptococcus	Neisseria family	Clostridia family	Enterobacteriaceae family, Yersinia	-	Coiled shaped

Table 2: pH of the selected juices.

S. No.	Juice sample	pH
1.	Strawberry	7.54
2.	Apple	4.7
3.	Pomegranate	3.94
4.	Guava	4.54
5.	Apple	3.91
6.	Grapes	3.81
7.	Mango	4.45

Spirilla were only observed in mango juice the rest of the samples were resistant to its growth (Table 1).

The pH of the selected fruit juices are shown in Table 2. The strawberry milk had nearly neutral pH while the rest of the juices have their pH on acidic side.

The bacteria always grow in suitable environment like proper temperature, pH of the medium and availability of nutrients. The pH and nutrition value of the branded fruit juice are different. On this basis various bacteria grows which leads to their spoilage which means that fruit juice provides a good media for the growth of bacteria. In this way branded fruit juice loses their nutritional value and can cause health abnormalities. The juice makers claim that these are properly sterilized and packed. However, the presence of these bacteria showed that the medium was not a properly sterilized one. Therefore, fruit juices makers should be aware that the process of fruit juice extraction and packing must do with care which will reduce the chances of contamination of bacteria. In this way the rate of diseases caused by bacteria can be reduced.

### CONCLUSIONS

The microbial study for branded fruit juices was conducted and various kinds of bacteria were detected. The bacteria which were observed are *cocci* in large

quantity, *bacilli* in moderate amount and *spirilla* in minute quantity. The bacteria always grow in suitable environment like proper temperature, pH of the medium and availability of nutrients. When contaminated branded fruit juice loses their nutritional value and can cause health abnormalities. The juice makers claim that these are properly sterilized and packed. However, the presence of these bacteria showed that the medium was not a properly sterilized one.

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