# "Studies On Microbial And Metallic Analysis Of Fruit Juices"

Dr. Varsha U. Lomate

Assistant professor, Academic Staff College, Sant Gadge Baba Amravati University, Amravati

### Miss Anjali Jiraffe

Ex- P.G Student Dept. of Microbiology Sant Gadge Baba Amravati University, Amravati

Abstract: Fruit juices are well recognized for their nutritive value, mineral and vitamin content. They are nutritious beverages and can play a significant part in a healthy diet because they offer good taste and variety of nutrients found naturally in fruit. But now a days chance of contamination of fruit juice get increases due to many condition. Most of the reported fruit juice borne outbreaks have been associated with bacterial contamination, particularly members of the Enterobacteriaceae. Of these, Salmonella and Escherichia coli in sprouted seeds and fruit juices are of particular concern. Outbreaks linked to protozoa e.g. Cryptosporidium, Cyclospora, Giardia etc have been associated more with fruits than with vegetables.

Keywords: analysis, contamination, fruit, fruit juices, microorganism

#### I. INTRODUCTION

Juice is the liquid that is naturally enclosed in fruit or vegetable tissues. It is prepared by mechanically squeezing or macerating fruit or vegetable without application of heat or solvent and which are nutritive, invigorating and nonalcoholic beverages beverage, which is very popular throughout the world. It is important source of bioactive compounds such as phenolics (e.g. Flavanone glycosides hydroxgannamic acids).

Vitamin C and carotenoid which is an excellent source of bio available antioxidant phytochemical and it also helpful to improve blood lipid profiles especially the people affected with hypercholesterolemia <sup>[1]</sup>.They are very scrumptious and palatable and they have most of the minerals like calcium, magnesium, phosphorus and vitamins<sup>[2]</sup>. Food security is a complex issue which is influenced by a number of factors.

The constituents of processed juices are mainly water, sugar, preservatives, color and fruit pulp. The most commonly used preservatives are benzoic acid, ascorbic acid or sulphur dioxide. Natural colors such as Acid is an essential universal constituent of juice and the most commonly used is citric acid. Most fruit juices contain sufficient nutrients that could support microbial growth.

Several factors encourage prevent or limit the growth of microorganisms in juices, the most important are pH hygienic practice and storage temperature and concentration of the

preservative storage of products at refrigerator temperature or below is not always best for the maintenance of desirable quality of some fruits. Water used for juice preparation can be a major source of microbial contaminants such as total coliforms, fecal coliform fecal streptococi etc.Environmental fomites may also make the fruits unsafe and spread of Salmonella, Shigella, Vibrio, Eschericha coli and other cause disease as well fruit spoilage<sup>[3]</sup>. Spoilage yeasts such as Candida and Saccharomyces cervisiae, lipolytica Zygosaecharomyces spp. can tolerate acidic environments. It should also be noted that changes in pH could transform a food into one which can support the growth of pathogen.

Fruit juice and drinks are nutritious which offer great taste and health benefits. Most fruits juices and drinks brought from grocery and supermarket shelves are pasteurized. However a small percentage of juices are unpasteurized. This means that there is a chance the product may contain bacteria harmful to our health. Unpasteurized fruit and drinks have posed serious health risks in recent years.

Fruit Juices are an important source of energy with the carbohydrates contained. These are the indispensable nutrients for the children, people belonging to the old age for the minerals and vitamins in particular fruit. The vitamins have vital importance for our bodies and are not synthesized in our bodies, which should be taken from outside have important contribution in many chemical events which are formed in our cells. The vitamins are separated into two groups as vitamins which melt in fat as (A,D, E and K and which melt in water as B group vitamins B1, B2, Niacin, Pentatonic acid, B6, Folic acid, Biotin and Vitamin C. In recent years the increasing consumer awareness has emphasized the need for microbiologically safe food. Since the human food supply consists basically of plants and animals or products derived from them, it is undesirable that food supply can contain microorganisms in interaction with the food. When the microorganisms involved are pathogenic, their association with food is critical from public health point of view.

The range of microorganisms associated with outbreaks linked to fresh product encompasses bacteria, viruses and parasites. The products of most concern are sprouted seeds and unpasteurized juices. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the *Enterobacteriaceae*. Of these, *Salmonella* and *Escherichia coli* in sprouted seeds and fruit juices are of particular concern. Outbreaks linked to protozoa e.g. *Cryptosporidium, Cyclospora, Giardia* etc have been associated more with fruits than with vegetables. Protozoa and viruses are most often associated with contaminated water or food handlers. Fruits and vegetables normally carry a nonpathogenic epiphytic microflora<sup>[4]</sup>.

Pesticide residues, crop contaminants (aflatoxins, patulin, ochratoxin, etc.) naturally occurring toxic substances and heavy metals are the major contaminants found in fruit and vegetables. Pesticides are used in management of pests and diseases in Agricultural and Horticultural crops. Heavy metals are present in the irrigation water and other manures. Infested seeds, irrigation water and soil act as the source of the fungal toxins.

Pesticides can leave adverse effects on the nervous system. Some harmful pesticides can cause several hazardous diseases like cancer, liver, kidney and lung damage. Certain pesticides can also cause loss of weight and appetite, irritability, insomnia, behavioral disorder and dermatological problems. The pesticide residue found in fruit and vegetables include residues of both banned (Aldrin, Chlordane, Endrin, Heptachlor, Ethyl Paration, etc) and restricted pesticides for use in India (DDT, Endosulfan, etc). Heavy metals also cause adverse effect in human metabolic system, skin diseases, heart problems, etc.

Serious health hazards due to presence of pathogenic microbes in food can lead to food poisoning outbreaks<sup>[5]</sup>. In India the chance of transmissions of disease through fruits juices are due to unsatisfactory hygiene and adulteration practices. Microorganisms are present both inside as well as outside of fruit. At the time of consumption majority of bacteria found on surface are usually Gram negative and belong to the *Enterobacteriacae*. Many of these organisms are usually non pathogenic to human. The inner tissues of fruit are usually regarded as sterile. However, bacteria can be present in low number as a result of the uptake of water through certain irrigation or washing procedures.

Fruit and fruit juices are commonly contaminated with bacteria, and contamination also caused by damaging of fruits due transporting process. Use of unclean water for dilution, dressing with ice prepared by using contaminated water, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can act as sources of contamination. Such juices have been found to be potential sources of bacterial pathogens notably *E. coli* 0157:H7, spp. of *Salmonella*, *Shigella* and *S. aureus* <sup>[6]</sup>. Flavoring agent, water and other chemical are all major sources of microbial contamination.

Fruit juices spoilage is caused by limited number of yeast, mould and acid tolerant bacteria. Infection of fruit Juice commonly occurs via raw material, returned bottles. Insects are increasingly recognized as a vector for yeasts many insects carry yeasts and insect grass, notably from fruit juices. Although many of 800 or 50 yeasts discovered have been found in soft drink and fruit juices.<sup>[7]</sup>. Acetobacter, Bacillus, Clostridium, Gluconobacter, Lactobacillus, Leucunostoc, Saccharabacter, Zymobacter and Zymomonas, Glucconabacter is common spoilage agent of fruit juice.

The quality of fruit is strictly maintained in developed countries under several laws and regulations but in many developing and undeveloped countries, the manufacture is not concerned 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. Fruit juices are available in essentially the same form almost anywhere in the world. From polar bases to the tropics and from the largest developed countries, fruit juices are available in bottles, cans, laminated paper packs, pouches cups and almost every other form of packaging known<sup>[8]</sup>.

In recent years these juices have been included significantly in the diet of most people, irrespective of age. Therefore maintaining the quality of processed fruit juices is an important concern. In order to develop awareness among the people about fruit juices, this study attempts to measure nutritional and microbiological quality of industrially processed locally available fruit juices.

The sources of these metals in food crops include discharges of these metals of waste having heavy metals onto agricultural lands, wastewater from different industries and sewage, application of some pesticides, metal aerosol deposition from the atmosphere due to emissions from vehicles and other sources. These trace metals are also translocated from the soil solution through the roots to other parts of the plant.

Cultivation of Citrus crops is a major source of income for many farmers in the district. Also the leading exporter of lime juice in Ghana and some citrus processing factories obtain their raw materials from the district. Accumulation of these heavy metals in the crops in higher concentrations could cause serious risk to human health if the crops are consumed and also adversely affect export. On this background therefore in the present research work the levels of As, Pb, Cr, Ni, Cu, Zn and Fe in orange, lime and lemon fruits grown in the Abura Asebu Kwamankese District in Ghana are determined. The concentrations of the various metals determined in this study are compared with recommended dietary references values for food energy and nutrients levels in other countries.

### II. MATERIAL AND METHOD

This research work is based on the routine microbial analysis process using specific media.

- A. MICROBIAL ANALYSIS OF DIFFERENT FRUIT JUICE SAMPLES IS CARRIED OUT (N=10) SAMPLES SELECTED FROM DIFFERENT AREAS OF AMRAVATI CITY ARE AS BELOW.
- ✓ Pineapple Juice (Rajkamal)
- ✓ Pineapple Juice (Gadge nagar)
- ✓ Mosambi Juice (Rajkamal)
- ✓ Mosambi Juice (Gadge nagar)
- ✓ Grapes Juice
- ✓ Apple Juice
- ✓ Mango Juice(Biyani Square)
- ✓ Mango Juice(Gadgenagar)
- ✓ Orange Juice (Rajkamal)
- ✓ Orange Juice (Gadge nagar).
- ✓ Pomegranate juice

Total no of microorganisms are counted by Standard Plate Count Method (SPC)

Total coliform number is counted by Most Probable Number Method (MPN)

*E. coli* is isolated by Presumptive Test, Confirmed Test and Completed Test

## B. METALLIC ANALYSIS OF FRUIT JUICE

For the metallic analysis of fruit juice Atomic Absorption Spectrophotometer is used.

### TYPICAL ANALYTICAL PROCEDURE

Sample Preparation



20ml of fruit juice in 100ml volumetric flask

# $\int$

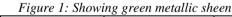
Addition of 10ml HCl then make up vol. With H<sub>2</sub>O shake well



Centrifugation or filtration of Sample to remove solid particle

### III. RESULT AND DISCUSSION

In the presumptive test positive result showed changes in broth color. The purple broth turns yellow it indicated that acid formation in the test tube and gas formation in durham's tube. In the second step i.e. confirmed test, for the confirmation of the positive result, loopful culture inoculated on the EMB agar and it is incubated for 24 h at  $37^{0}$ c. After incubation opaque green metallic sheen colonies were observed on EMB agar plate (fig 1). In the third step i.e. completed test it also shown the positive result. Pineapple Juice samples shown high number of coliforms as compared to other juice sample. Apple Juice sample shown less number of coliforms. The morphological characters and biochemical test were also performed.



Sr.No.	Sample	MPN	SPC
		Coliforms/100ml	
1	Pineapple (A)	32	$30x10^{3}$
			CFU/ml
2	Pineapple (B)	34	$28 \times 10^{3}$
			CFU/ml
3	Mosambi Juice	28	$21 \times 10^{3}$
	(A)		CFU/ml
4	Mosambi Juice	32	$30x10^{3}$
	(B)		CFU/ml
5	Grapes Juice	24	$30x10^{3}$
	_		CFU/ml
6	Apple Juice	19	$17 \text{x} 10^3$

			CFU/ml
7	Mango Juice	34	$25 \times 10^3$
	(A)		CFU/ml
8	Mango Juice	36	$25 \times 10^3$
	(B)		CFU/ml
9	Orange Juice	26	$21 \times 10^{3}$
	(A)		CFU/ml
10	Orange Juice	26	$19 \times 10^{3}$
	(B)		CFU/ml
11	Pomegranate	23	$15 \times 10^{3}$
	Juice		CFU/ml

Table 1: Showing results of MPN and SPC of Fruit JuiceSamples

### METALLIC ANALYSIS

Absorption spectrophotometer method is used for metallic analysis of the Fruit Juice sample. In the metallic analysis, the concentration of Cd, Pb, Cu, Fe were detected from all sample. For the analysis the concentrated HCl is used. In all Juices of concentration of Pb is very less.

Sr.	Sample	Cd	Pb	Cu	Fe
No.	_				
1	Pineapple	0.024	0.011	0.039	0.093mg/L
	(A)	mg/L	mg/L	mg/L	
2	Pineapple	0.023	0.011	0.039	0.093mg/L
	(B)	mg/L	mg/L	mg/L	
3	Mosambi	0.019	0.009	0.031	0.083mg/L
	Juic(A)	mg/L	mg/L	mg/L	
4	Mosambi	0.018	0.009	0.031	0.071mg/L
	Juic(B)	mg/L	mg/L	mg/L	
5	Grapes Juice	0.021	0.007	0.033	0.099mg/L
		mg/L	mg/L	mg/L	
6	Apple Juice	0.022	0.006	0.033	0.071mg/L
		mg/L	mg/L	mg/L	
7	Mango	0.019	0.010	0.033	0.071mg/L
	Juice (A)	mg/L	mg/L	mg/L	
8	Mango	0.019	0.009	0.037	0.098mg/L
	Juice (B)	mg/L	mg/L	mg/L	
9	Orange	0.018	0.007	0.020	0.101mg/L
	Juice (A)	mg/L	mg/L	mg/L	
10	Orange Juice	0.016	0.007	0.020	0.101mg/L
	(B)	mg/L	mg/L	mg/L	
11	Pomegranate	0.023	0.009	0.037	0.098mg/L
	Juice	mg/L	mg/L	mg/L	

Cd-Cadmium, Pb-Lead, Cu-Copper. Fe-Iron Table 2: Showing result of metal analysis of Fruit Juice Sample

### IV. CONCLUSION

The MPN analysis showed contamination in road side fruit juice samples collected from different areas of Amravati city (table1). This would be possible of the poor quality of water used in juice preparation. Moreover water is one of the major Sources of fruit juice contamination.

Water and unsatisfactory hygiene is responsible for contamination of these fruit juices along with it insects and road side soil are major source of contamination. Regular monitoring of road side fruit juices is essential to avoid further complication with respect to food borne illness

From the results of the present study, it is recommended that road side fruit juice sellers must use potable drinking water to avoid contamination of fruit juice.

### REFERENCES

- Franke AA, Cooney RV, Henning SM and Custer LJ, (2005). Bioavailability and antioxidant effects of orange juicecomponents in humans. J Agric Food Chem 53 (13): 5170–8.
- [2] Applied FDA, (1999). Fruit morphology and composition. Center for Food Safety and Nutrition United States Food and Drug Administration. Available at: http://vm.cfsan.fda.gov/~comm/ juicback.html. Accessed 06 August (3); 185-192.
- [3] Doyle MP, Beuchat LR & Montville TJ, (2001).Food Microbiolog,vol.5,23-27.
- [4] Ray B, (2004); Fundamental Food Microbiology. Third edition. Londan: CRC Press
- [5] Bagde NI and Tumane PM, (2011).Studies on microbial flora of fruit juices and cold drink. Asiatic J of Biotech. Res,2(04) :454-460.
- [6] [6] Buchaman RL, Edelson SG, Miller RL and Sapers GM, (1999). Contamination of intact apples after immersion in an aqueous environment containing Escherichia coli O157:H7. J. Food Prot. 62:444-450
- [7] Jayalakshmi T, Krishnamoorthy P, Kumar RG and Sivamani P, (2011). The microbiological quality of fruit containing soft drinks from Chennai. J. Chem. Pharm. Res. 3(6):626-630.
- [8] Tasnim F, Hossain MA, Nusrath S, Hossain M and Lopa D, (2010). Quality assessment Of Industrially Procssed Fruit Juices Available in Dhaka City,Bangladesh.Mal J.Nutr 16(3):431-438.