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Invitro Evaluation of Microbial Content in Bottled Soft Drinks, Fruit Flavored Juices and Fruit Juices

J. Ishwarya*[1], D. Prema[2]

[1]Assistant Professor, [2]Research Scholar,
[1,2]PG and Research Department of Microbiology,
Kamban College of Arts and Science for Women, Tiruvannamalai, Tamil Nadu, India

ABSTRACT

The soft drinks, fruit flavoured juices and fruit juices are the fastest growing and rapidly changing areas in the food and drink industry. From high source of energy to no / low sugar, functional to long life, carbonated or still, soft drinks and their associative preservative and packaging systems have their own microbiological issues. Many microbes found in soft drinks fruit flavoured juices has environmental or raw material contaminants, but relatively few can grow in the acidic or low oxygenic environment. Spoilage will be seen as the growth and production of metabolic by products, produced by the microorganisms present in that drinks. Most spoilage is caused by yeasts and mold species and some of the spoilage is caused by bacteria. Bacteria that cause spoilage in the soft drinks industry they include *Acetobacter, Alicyclobacillus, Bacillus, Clostridium, Gluconobacter, Lactobacillus, Leuconostoc, Saccharobacter, Zymobacter* and *Zymomonas. Gluconobacter* is a commonest bacterial agent of fruits. *E.coli, Salmonella, Klebsiella* and *Vibro* have been isolated from soft drinks, fruit flavoured juices and fruit juices. The aim of this study is to evaluate the microbiological load of soft drinks and fruit juices in invitro condition.

KEYWORDS: *soft drinks, fruit flavoured juices, bottled fruit juices, microbes, spoilage, identification*

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INTRODUCTION

A soft drink is a drink that usually contains water, sweetener and natural or artificial flavoring substances. The sweetener may be sugar high fructose corn syrup, fruit juice or combination of these. Soft drinks and packed fruit juices may also contain caffeine, colorings, and preservatives.

Soft drinks may be served cold, over ice cubes or at room temperature. They are available in many container formats; cans, glass bottles and plastic bottles, fruit juices and soft drinks are an important products market with the food industry. The increase in variety of products released at a bewildering rate will alter the potential for spoilage problems.

The soft drinks are generally poor in nutrients that are generally spoiled by few microorganisms usually contaminated with yeast and few acid -tolerant bacteria and fungi. Infection in soft drinks occurs commonly by the raw materials returned from bottles or aerial vectors.

Many of these insects which carry yeasts and from fruit juices, are particularly rich in soft drinks. The term fruit containing soft drinks originated to distinguish non-alcoholic beverages from hard liquor or spirits. Fruit containing soft drinks are carbonated beverages, usually it contains sweetening agent, edible acids and natural or artificial flavors that is Soft drinks include cold beverages, fruit flavored drinks and ginger ale and root beer. The chemical -physical and composite characteristics of soft drinks make them susceptible to microbial spoilage. They are usually characterized by high C/N ratio and low PH, which allows the microbial growth such as acetic acid bacteria and lactic acid bacteria, mold and yeast.

Soft drinks contain water, 8-12 % w/v sweetener, 0.3-0.6% co2, 0. 05-0.3% acidulates, 0.1-0.5% flavoring

agents, 0-70ppm coloring agent lawful limits of preservatives (chemical) <100ppm antioxidants and foaming agents (Example: saponins up to 200mg/ml). However,certain ingredients may cause health hazards if consumed in large quantities, generally with regard to preservatives and sweeteners. On other hand there is a trend to produce wide ranges of more specialized soft drinks, there is also pressure to minimize the use of, in particular, artificial and synthetic additives and ingredients.

Modern soft drinks are classified in several ways on the basis of their sugar and fruiting juice content, flavoring, carbonate level ingredients and functionally most popular soft drinks are 1. Ready to drink essence flavored beverage, 2. Ready to drink beverages contain fruit or fruit juice, 3.Beverages ready to drink after dilution.

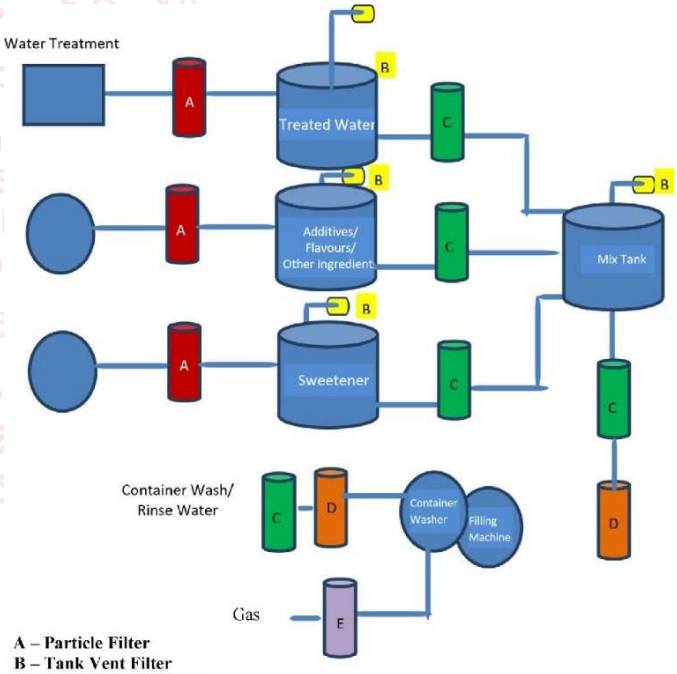
Nowadays there is a consciousness of health and wellness among the consumers and increasing importance given to fitness and healthy lifestyle choices. There is changing work and lifestyle habits which leave less time for home cooking and therefore demand on spur for convenience and "complete nutrition" from replacement in meals. There is a greater inclination to"self care" rather than medicate, a greater awareness of the functional benefits of health beverages and a greater willingness to pay a premium for such beverages. The non-carbonated beverages in the market of many countries with Rs.500crores (of market range) composed of fruit drinks, nectar and juices.

Microbial contamination occurs in the production process of soft drinks itself, it includes the factory environment, equipment and the raw materials and packages, and also lack of hygiene. The source of contamination can be packaging materials like cans and bottles. Primary spoilage is due to yeasts in carbonated products, due to their high carbonation resistance and low PH levels.

Some yeast moderately tolerate high carbonation and are able to grow in refrigerator temperature. Soft drinks easily contaminate with white, delicate, fluffy, cottony masses that are molds. During the process of production fungal spores or conidia or mycelium fragments easily contaminate the beverages. Some of the lactic acid bacteria can easily grow in soft drinks. Enteric pathogenic bacteria, viruses, and protozoans are responsible for the beverages related outbreaks.In many beverages related outbreak cases the causative agents are unknown.

High-grade bottled soft drinks enable the dweller in rural communities to enjoy a food product which a few years ago was obtainable only in towns and cities, directly from soda fountains. Flavours and condiments, well-known household articles, are used in soft drinks, and are of a varied nature designed to make the product attractive to the taste. In addition to being delectable, soft drinks have food value, due sometimes to their content of sweetening ingredient, which amounts to from 5 to 12 per cent of the total weight of the beverage, and, in some cases, tothe fruit extracts which they contain. The quality of bottled soft drinks depends largely upon the demand made by discriminating consumers. Some knowledge of the composition and preparationof these products for the market, as set forth in this article, should enable the average consumer to ask for only high-grade beverages. The annual consumption of bottled soft drinks in the United States prior to war restrictions in production is estimated as about three billion bottles. It is estimated that over 10,000 establishments, employing about 75,000 people, are engaged in the bottling of soft drinks in the United States.

FILTRATION PROCESS TO REMOVE MICROBE IN SOFT DRINK PRODUCTION



Microorganisms play a central role in the food spoilage and beverages, mainly with reduced water activity and also high acidity. The soft drink spoilage mainly depends on its position and the quality of ice used in that cool drink. The aim of this study was to evaluate the microbiological load of soft drinks and fruit juices in invitro condition.

MATERIALS AND METHODOLOGY

SAMPLE COLLECTION

Soft drinks and fruit containing soft drinks were obtained from various supermarkets. One third of the sample was transferred into sterile flask for further

processing the date of manufacture and the soft drinks natures were noted.

SAMPLE PROCESSING

Liquid samples were used.1ml sample transferred to sterilize test tubes containing 9ml of 0.1% peptone water mixed thoroughly by shaking and serially diluted.

ISOLATION OF BACTERIA FROM SOFT DRINKS SAMPLE SERIAL DILUTION

One ml of packed juice sample was taken into a 10^{-1} tube containing 9 ml of saline. From this, 1 ml of sample was serially diluted 10^{-2}, 10^{-3}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6},10^{-7}, 10^{-8}. 1 ml was taken from each tube and inoculated into nutrient agar plates andspread using L-rod. Plates were incubated at 37ºC for 24 hours. Colony morphology was observed and the isolated colonies were inoculated into nutrient broth and incubated at 37ºC for 24 hours. This test sample was further used to identify the morphological and biochemical characteristics of the organisms.

PLATING METHOD

Pour plate method is carried out to determine the total viable count usingtotal plate count agar for soft drinks and fruit juices. Different types of selective media used for isolation and identificationbacterial colonies streak plate method.

MAINTAINENCE OF CULTURE

- Macconkey agar used for the isolation of Gram negative enteric bacteria.
- SS agar for their isolation of *Shigella* and *Salmonella*.
- TCBS agar for the isolation of *Vibrio.*
- EMB agar used for isolation of *Escherichia coli* and *Proteus*.
- Nutrient agar plates and slants used for the maintenance of all cultures.

IDENTIFICATION OF BACTERIA

The organisms were identified by the morphological characters and biochemical characters. The Morphological characteristics were identified by the following methods Gram Staining, Capsule Staining, Motility Test. The biochemical tests were conducted by the following methods as described bycappuccino and Sherman (1999) to identify the bacteria.

QUANTITATIVE ESTIMATION

MPN Method for estimation of coliforms using three test tubes where inoculums were 10, 1, 0. 1ml. Macconkey broth and Brilliant green lactose bile broth used for the procedure.

RESULT AND DISCUSSION

Microbiological examination of soft drinks and fruit drinks for the Gram staining and the motility test shows the test result. The organism is subjected to biochemical tests it shows the results.After 24 hours of incubation, determination of the MPN test estimated the total coliforms using the MPN chart. On TCBS agar the organism produces yellow and green colonies to be considered as *Vibrio*. On SS agar organisms produce colorless, transparent with black precipitation on the center of colonies it to be considered as *Salmonella*. On EMB agar the organism green metallic sheen colonies to be considered as *Escherichia coli.* On Macconkey Lactose fermenting colonies observed as organisms considered to be *Klebsiella* .

In this study it clears that all the juices and soft drinks contain a significant amount of microorganisms. The viable count of microbial load in total showed that 0.7×10^{3}-9×10^{8}cfu/ml. The total coliform presence in all the drinks implied that negative relation with quality and safety of food. 2.55×10^{6}-9×10^{8}cfu/ml of coliforms count in drinks were identified and determined.

The contamination might be due to raw materials which were used in the production. The fruit juice or soft drinks with low PH shows the limited number of organisms. The microbial loadin food can be linked with a large number of risk factors such as improper processing and improper handling, which may be due cross contamination with water, washing, dilution, rotten fruits, daily utensils, knife, flies and also ice which was added to the drinks during preparation.

Most of the gastroenteritis diseases cause strains are isolated from the drinks, it causes food poisoning like vomiting and diarrhea which results in extreme dehydration and if not treated leads to death. To prevent such contaminations in fruit juices and soft drinks with a Good Hygiene Practice (GHP) and HACCP on the basis of food safety production of government healthagencies. They must adopt some measures to educate the workers about food safety and hygiene practices and to develop a regular monitoring system and improved surveillance system on food borne pathogens.

IDENTIFICATION AND TOTAL VIABLE COUNT BACTERIAL ISOLATES FROM SOFT DRINKS

S. No	Colony Morphology	Gram Staining	Motility	I	MR	VP	CIT	CAT	OXI	UR	TSI	Organism	Viable Count cfu/ml
1	Black colonies	(-)ve rod	+	-	+	-	-	+	-	-	+	*Salmonella*	7.0×10^{3}
2	Yellowish green colonies	(-)ve rod	+	+	-	+	+	+	+	-	+	*Vibrio*	6.9×10^{4}
3	Metallic sheen colonies	(-)ve rod	+	+	+	-	-	+	-	-	+	*E.coli*	9×10^{8}
4	Lactose fermenting pinkcolonies	(-)ve rod	-	-	-	+	+	+	-	+	+	*Klebsiella*	2.55×10^{8}

CONCLUSION

Easily availability with low price made the drinks highly popular to the common people. But these drinks will be beneficial if these drinks are contamination free. The current study revealed that soft drinks and fruit juices contain microbial load. To avoid the spoilage cases of soft drinks in this work stresses the need for investigations very deeply on the certain substances' role in their potential control of microbial proliferation. The failure of the traditional antimicrobial substances such as weak acids can be counteracted by the presence of specific compatible molecules with all the characteristic products. In addition, such a strategy can meet the demands of consumers for avoiding chemical preservatives, which can besubstituted by a highly accurate and balanced aroma compounds addition. Regular monitoring of quality checks in the soft drinks, highly recommended for human consumption and it avoids any outbreak of bacterial pathogens in future.

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