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The Microbiological Quality of Fruit Containing Soft Drinks from Chennai

T. Jayalakshmi *[1], P. Krishnamoorthy[1], G. Ramesh Kumar[2] and P. Sivamani[3]

[1]Department of Bioinformatics, Bharath University, Chennai, Tamilnadu, India
[2]Department of Bioinformatics, MIT Campus, Anna University, Chennai, Tamilnadu, India
[3]Microlabs, Vellore, Tamilnadu, India

ABSTRACT

The soft drinks sector is one of the fastest growing, most innovative and rapidly changing areas of the food and drink industry. From high energy to no / low sugar, functional to long life, carbonated or still, soft drinks and their assosiative preservative and packaging systems have their own microbiological issues.many have adapted to growing in the unusual environment within a beverage factory and can be extremely difficult to remove once established. Many micro organisms are found in soft drinks as environmental or raw material contaminants, but relatively few can grow within the acidic or low oxygen environment. Yeasts are the most significant group of micro organisms associated wih the spoilage of soft drinks and fruit juices. Spoilage will be seen as the growth and production of metabolic by products, e.g co_2, acid and tainting compounds. Most spoilage is therefore by yeasts and mold species, with yeasts most important and some spoilage is by tolerant bacteria. Bacteria that has been associated with spoilage in the soft drinks industry include Acetobacter, Alicyclobacillus, Bacillus, Clostridium, Gluconobacter, Lactobacillus, Leuconostoc, Saccharobacter, Zymobacter and Zymomonas. Gluconobacter is a common agent of fruits. It is a strict arobe requiring free oxygen. E.coli and Enterococci have been isolated from citrus juices, apple juice and have been associated with Cryptosporiadiosis. Contamination by hepatitis A and Norwalk like viruses have been reported in the fruit juices. The aim of this study is to isolate and identify the microorganism from the soft drinks.

Key words: soft drinks, microbes, spoilage, identification.

INTRODUCTION

A variety of soft drinks are being presently produced in the country, e.g. sweetened carbonated (aerated) soft drinks, still beverages containing fruit juice/ pulp and soda water. Among these, the share of fruit juice based beverages is presently quite small as compared to synthetic carbonated drinks. Gradually there is a distinct shift towards fruit juice based beverages for obvious advantages of the higher nutritional value over the synthetic aerated waters. Due to their low pH, soft drinks constitute a hostile environment in which the great majority of microbes die, although Escherichia coli O157 and Salmonella species can persist for weeks in chilled, fruit juices [2,4]. Spoilage of soft drinks is caused by a limited number of yeasts, moulds and acid-tolerant bacteria. Spoilage effects include formation of clouds, particulates, taints and excessive gas [1]. Infection of softdrinks commonly occurs via raw materials, returned bottles or aerial vectors [3]. Insects are increasingly recognized as a vector for yeasts. Many insects carry yeasts and insect frass, notably from fruit juices (Drosophila sp.), is particularly rich in soft-drinks spoilage yeasts [5,6]. Although many of the 800 or so yeasts discovered hitherto have been found in soft drinks or fruit juices [7], relatively few species can grow in this environment or cause spoilage [8]. The term fruit containing soft drink was originated to distuinguish non alcoholic beverages from hard liquor or spirits. Fruit containing soft drinks are alcoholic carbonated or non carbonated beverages, usually containing a sweetening agent, edible acids, and natural or artificial flavours soft drinks include cold beverages, fruit flavoured drinks, ginger ale and root beer.In general the term is used only for cold beverages. Hot chocolate, tea and coffee are not considered soft drinks, and is still commonly used in this manner. Today there is a growing health and wellness consciousness among consumers and an increasing importance given to fitness and healthy lifestyle choices. Changing work and lifestyle habits leave less time for home cooking and therefore spur demand for convenience and ‘complete nutrition’ from meal replacements. 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 [9]. The Rs 500 crore non-carbonated beverage market in the country is composed of fruit drinks, nectar and juices. Microbes play a central role in the spoilage of foods and beverage, mainly those with high acidity and reduced water activity. The spoilage of the soft drinks depends on its composition and the quality of ice used to cool drinks, 9% contained Escherichia coli or coli forms, 1% Enterococci and the microbiological quality of ice depend on the type of use, the type of premises, and the type and place of production.

EXPERIMENTAL SECTION

Sample collection

Fruit contain soft drinks were obtained from Chennai. Among that soft drinks available in the market , One third was transferred into a 500ml sterile flask for further processing. Care was taken to note the date of manufacture and the nature of soft drinks.

Methods

The samples were processed to identify the type of organisms and the number of organisms by the following three techniques.

- Standard Plate Count Technique(SPC)
- Most Probable Number Technique(MPN)

- Membrane Filter Technique(MFT)

Isolation and identification of bacteria

Isolation of bacteria from the soft drinks sample

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

Enumeration of Bacterial isolates

The number of individual colonies on an agar plate were counted using Colony Counter.

Number of CFU/ml of packed juices = Average number of colonies per plate/vol. of sample x dilution factor.

Morphological characterization studies, such as Gram staining, Motility Test i.e. Hanging Drop method is carried out [10].

Biochemical Reactions

After the morphological characterization studies, the test samples were further identified by using biochemical reactions by doing IMViC. The organisms can be identified by IMViC (Indole, Methyl red, Voges Proskauer and citrate utilization test) and TSI confirmed by doing tests such as starch hydrolysis, catalase test, TSI test, Urease test etc.

RESULTS

Microbial analysis of soft drinks for gram staining gave blue colour confirmed the presence of gram positive cocci. The motility test conducted for gram positive showed the absence of flagella which is non-motile.

Gram positive cocci were further subjected to biochemical tests. Indole test gave a negative result showed the absence of tryptophan. For Methyl Red, positive result was obtained showing the ability of cocci to oxidize glucose. Negative results were abtained for voges-Proskauser test at the end of 48 hrs showed the absence of acetoin.

Negative results was obtained for citrate utilization and was due to the inability to ferment citrate at the end of 24 hrs. The urease and starch hydrolysis test showed a negative result which showed the absence of urease and exo-enzyme amylase at the end of 24 hrs. positive results for catalase test and sugar fermentation confirmed the presence of Gram positive cocci, *Staphylococcus aureus.*

Identification ofbacteria from fruit juices:
Sample: Soft drinks
Organism isolated and identified: *Staphylococcus aureus*
Gram Staining: Gram positive cocci
Motility test: Non-motile
Biochemical Reactions:
1. **Indole:** Negative
2. **Methyl red:** Positive
3. **Voges proskauer:** Negative
4. **Citrate test:** Negative
5. Urease test: Negative
6. Catalase test: Positive
7. Starch test: Negative
8. Sugar fermentation test: Positive

Figure: 1 shows the Staphylococcus aureus in agar medium

DISCUSSION

Soft drinks are spoiled by microbes like bacteria and fungi. The pH of soft drinks is low due to its ingredients like acid. Soft drinks consists number of acid tolerant or acidophilic species like Lactobacillus and Leuconostoc.

The diversity of lactic acid bacteria in a variety of food releated ecosystems was assessed. The microbes were present in traditional fermented foods and in spoiled beverages. Acid toleant strain were predominantly isolated from traditional fermented beverages.

Microbiological analysis of fruit containing soft drinks revealed the presence of bacteria such as Staphylococcus aureus and klebsiella pneminiae.

Dominant lactobacillus species present in soft drinks are Lactobacillus acidophilus.

Recently, molecular technique such as nucleic acid and antibody based assays such as polymerase chain reaction are used to analyse microbes easily in fruit containing soft drinks in

large scale industries which are highly sensitive,selective,rapid and reliable conventional techniques o detect pathogenic microbes.

CONCLUSION

- Isolation and identification of microbes on fruit containing soft drinks were studied because soft drinks contains low pH and they are heat resistant.
- The number of organisms present in sample decreases when the dilution factor increases. By serial dilution technique, colony forming unit per ml were studied.
- Spread plate count technique(SPCT) and streak plate method was carried out to obtain the isolated colonies from the mixed cultures at the end of 24 hrs and it was sub-cultured on nutrient broth.
- The specific isllated colonies were subjected to gram staining and motility techniques from the nutrient broth within 12 hrs.
- Gram staining procedure was carried out to differentiate both gram positive and negative organisms based on morphological characteristics.
- Motility test was carried out to identify motile and non-motile organisms to confirm the presence or absence of flagella.
- Identification of the particular isolates were carried out by doing bio-chemical reactions for all samples at the end of 24 hrs.
- Confirmatory tests such as urease test,catalase test and sugar fermentation test were carried out to confirm the presence of particular species of that organism within 24 hrs of incubation.

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