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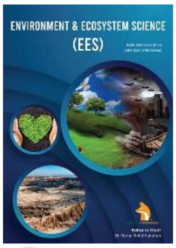

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RESEARCH ARTICLE

PHYSICO-CHEMICAL AND BACTERIOLOGICAL ASSESSMENT OF PACKED JUICES CONSUMED IN THE CAPITAL TERRITORY OF ISLAMABAD, PAKISTAN

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ABSTRACT

Modern soft drinks constitute a variety of different products. The high temperature in most nations necessitates a substantial intake of liquids to compensate for the predicted losses from respiration. Fruit juices aid detoxification in humans by utilizing acid, which is an essential universal ingredient of juices. Citric acid is the most widely used acid in juices. Physicochemical and microbiological assessments of packed juices have been conducted in the Chemistry Lab of Bahria University Islamabad. A set of eleven locally manufactured packed juices and nine imported juices samples were collected from four different shopping malls and roadside shops in Islamabad. All the twenty samples were quantitatively analyzed for their microbial contamination and physicochemical properties. First, the samples were subjected to the microbial analysis where they were tested for the total bacterial count, total coliform count, and salmonella and shigella count using Nutrient agar, EMB agar, and S.S agar, respectively. All the collected 20 juice samples were examined separately and individually and then compared with water quality standards given by World Health Organization (WHO) and PAK-EPA. After microbiological assessment of juice samples, they were further analyzed with physical and chemical parameters. Physical parameters such as pH, salts, temperature, electrical conductivity, etc. of the juices have been tested. Chemical parameters such as chlorides and alkalinity of the juice samples have been tested. After the testing, the results have been noted down and further calculations have been generated.

KEYWORDS

Bacteria, Degree Celsius, food poisoning, Molarity, Oxidative Reduction Potential, permissible limit, soft drinks.

1. INTRODUCTION

Modern soft drinks include a wide range of items. They can be classified in a variety of ways depending upon their characteristics for instance, based on the amount of sugar and fruit juice present, flavoring, carbohydrates, non-watery ingredients, etc. Available juices are rich in nutrients, vitamins, and minerals that contribute to good health. Orange juice contains a high concentration of vitamin C and antioxidants phytochemicals and substantially improves blood lipid profiles in hypercholesterolemic patients (Franke et al., 2005). Contamination of fruit and fruit juices by yeasts and molds often occurs in fallen or damaged fruit and these should be avoided if at all feasible. Microbial contamination can be found in water and other substances. Process machinery and filling lines are the most troublesome, and careful cleanliness is required. Mould deterioration in the factory is linked to inadequate cleanliness. Heat-resistant spores of many kinds can be generated, including ascospores, chlamydospores, and sclerotia. Many of the molds mentioned above can be detected on fruits both before and after harvest. Juices kept at different temperatures (27^{o}C and 40^{o}C) were tested for yeast and mold development. Some fungus has been discovered in fruit juices in Egypt. Microscopy of fungal isolates collected from fruit juice samples revealed the presence of the following species: Byssochlamys Nivea, Byssochlamys fulva, Neosartoryaficsheri, Penicillium italicum, Aspergillus flavus, and Talaromyces macrospores packed in Tetra Pack.

1.1 Quality and Safety Issues of Juices

Mostly the quality of juices is depending on their raw material and ingredients quality including water, fruits, sweeteners, `flavors, preservations, etc., and more focused on raw material or ingredients. As to produce superior quality products and premium quality of raw fruits and free from contamination and injuries are required. Additionally, these should not be affected by microbial hazards for instance yeast, mold presence, or the existence of any pathogenic microorganisms that will deal with the final quality of the juice and safety of the products (Ashurst et al., 2017). In the last two decades, fruit juice-borne disease decomposition

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problems have been reported due to the involvement of forborne pathogens including Escherichia coli, Salmonellas, and Cryptosporidium parvum, and also degeneration of microorganisms such as alicyclobacillus acidoterrestris (Cerny et al., 1984; CDC 1996, 1999, 2007). Furthermore, the problems related to some well-known fruits for example oranges and apples, have increased interest in exotic fruits like *Cupuacu* commonly known as Theobroma Grandiflorum, and *Acai* known as Euterpe Oleracea among others. It has been noted that due to the bioactive compound's existence in these fruits.

1.2 Bacterial Contamination in Juices

Fresh juices utilization has resulted in foodborne illness from other infective and pathogenic microorganisms such as *Escherichia* coli, *Salmonella*, *Shigella*, and *Staphylococcus aureus*. Mostly, illnesses caused by the fresh juices are conveyed from raw materials, processing conditions, improper handling, equipment, un-potable water, and also maximum storage at room temperature. Moreover, the disclosure of these juices into floating dust and unhygienic environments including dirty effluent water, debris, and garbage near to the food formation places and allows vectors such as houseflies to transference of microbes to the finished products. After the evaluation in literature, it has been reported that about 50 percent of microbial foodborne diseases are the cause of inappropriate food storage for instance ineffective chill storage and refrigeration management (Kaddumukasa et al., 2019).

1.3 Health Risks Associated with Microorganisms

A minute harmful microbe, mainly less than 1 percent of bacteria that can enter into our body and make us ill. These microbes are caused by flu and measles. In many of the studies, strong evidence is indicated that some microbes may contribute to other non-infectious chronic diseases for example some kinds of cancer, and coronary heart disease. Different types of microbes caused different diseases and these microbes that caused disease are called Pathogens.

2. Materials and Methodology

2.1 Sampling Location

The locally made juices samples were collected from different local shops at different spots of Islamabad capital of Pakistan while the international juices sample was collected from different supermarkets of Islamabad specifically from Shaheen Chemist (G-11), Al-Fatah Mall (F-8), Punjab Cash n Carry (G-9), Islamabad. Figure 1 shows a pictorial view of the juices.

2.2 Sample Size and Sample Collection

The sample collection was based on a comprehensive study based on consumer choice regarding juices sales in Islamabad. The brands consist of Nestle, Tops, Dayfresh, Maaza, Oolala, Spinta, Red bull, Eazu, Rani, Mogu, Fresh N Chill, Cappy Pulpy Orange, Fresher, Slice, Milo, Olpers, Vivo, Dewdrops, Nurpur. There were 20 samples in total.

Figure 1: Pictorial view of Juices samples

2.3 Microbiological Analysis

A standard method has been applied for microbiological analysis of individual juices to check the number of colony-forming bacteria, exist in the collected samples. The results of the analysis were expressed in the term CFU/ml (Yusufu et al., 2016). Before the starting of analysis of collected samples, all the apparatus was thoroughly washed and was autoclaved, along with all the prepared media, at 121°C for 30 minutes. Three agars were selected, to determine the total bacterial count, salmonella and shigella count, and total coliform count for each juice sample. Each agar was transferred into 30 sterile Petri dishes and incubated at 30°C for 24 hours. Afterward, 0.1 ml of the supernatant from each sample was poured onto the Petri dishes and thoroughly spread. Then the Petri dishes were properly sealed, labeled, and put in the incubator for 48 hours at 30°C. The entire process was carried out in the laminar flow to prevent any kind of external contamination (Agu, 2014).

2.3.1 Apparatus

Electronic weighing scale, Pestle, and mortar, Sterile plastic container, Spatulas, Refrigerator, Laminar Flow, Autoclave, Incubator, Centrifuge, Flasks, Funnel, Cotton Plugs, Measuring cylinder, Petri dishes, Test tubes, Pipettes, and Glass spreader.

2.3.2 Culture Media Preparation

A culture media is simply a medium, to grow different types of microorganisms and consists of the essential nutrients for microbial growth. For our analysis, three different media were prepared and used to determine specific microbial colonies.

2.3.2.1 Nutrient Agar

The nutrient agar is a general-purpose medium used for determining the total microbial count for different microorganisms. The process of agar was firstly mixing 12.6g of nutrient agar powder into the 450ml of distilled water and secondly, it autoclaved for 121°C for 30 minutes.

2.3.2.2 Salmonella and Shigella agar

The salmonella and shigella agar are used for determining the salmonella and shigella count. For preparing the agar, the mixture of 23.4g of the agar powdered was added into the 450 ml of distilled water and then autoclaved at 121°C for 30 minutes.

2.3.2.3 Eosin methylene blue (EMB) agar

The EMB agar is used for determining the total coliform count. The same process for agar preparation, firstly, the mixture of 15g of the EMB agar powder was poured into 400 ml of distilled water and then autoclaved at 121°C for 30 minutes.

2.3.3 Sample Preparation

Firstly, 0.9 mg of NaCl was added into 100 ml of purified water to prepare the normal saline solution, then autoclaved at 121°C for 30 minutes. 10 ml of this normal saline was poured into 30 sterile labeled test tubes for each sample. Then, 5 g of each juice sample was put in its assigned tube. The solution was then centrifuged for 10 minutes at 1000 rpm to obtain the supernatant (Giwa and Tasleem, 2012).

2.4 Physical and Chemical Assessment

Physical parameters such as pH, EC, Total Dissolved Solids (TDS), salinity, Dissolved Oxygen (DO), and temperature were measured instantly after opening the sample packet using the HACH Sention105 multiparameter. To begin, DO was measured to avoid the solubility of ambient oxygen. Total Soluble Solids (TSS) of orange juices were determined through a hand device refractometer (Atago, Japan) and it was corrected to the equivalent analysis at 20°C (AOAC, 1985).

2.4.1 Instruments used and analysis method

Physical parameters i.e., pH, EC, salt, temperature, TDS were investigated via multi-parameter tester 34. An electronic turbidity meter was used for examining the turbidity of the samples. The ORP process was done by Conductivity meter. Volumetric titration method was used for chemical analysis of the samples and microbiological parameters were determined by Spread plate method commonly used method and also by biochemical tests i.e., gram staining method

2.4.2 pH

The pH measures a solution's acid balance. It is defined as the logarithm negative of the concentration of hydrogen ions at base 10. The pH scale ranges between 0 and 14 which indicated very acidic to very alkaline, the value with pH 7 displays the neutral state of the solution. For clean waters, pH is controlled primarily by the equilibrium of CO_2, carbonate, and bicarbonate ions. Generally, natural waters have a pH range between 6.0 to 8.5, with lower values in diluted organic-rich fluids and higher values in eutrophic water, groundwater brines, and salt lakes.

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2.4.3 Total Dissolved Solids TDS

Inorganic and organic matter present in water that can pass through the filter of 2 microns is known as total dissolved solids (TDS). TDS will only tell how much the total amount of these ions are present in it but will not tell their relationship between them and nature.

2.4.4 Electrical Conductivity EC

Conductivity is defined as a function of water's ability to conduct an electric current. Distinctions in dissolved solids, typically mineral salts are very sensitive. Conductivity expressed in the unit as micro-Siemens per centimeter (μS cm-1) and, for a given water body, is associated with the concentrations of TDS and major ions.

2.4.5 Salt

The salty nature of water and the presence of dissolved salts is called saltwater or saline water. NaCl molecules get separated when salt molecules are dissolved in juices and then they become free ions.

2.4.6 Temperature

Soluble solids concentration (SSC) strongly affects the density of pineapple juice, and the juice is less affected by temperature. Different types of models were established as a function of SSC content and temperature.

2.4.7 Turbidity

Hazes and turbidity grow in apple juice due to the tendency of proteins, starches, and tannins to aggregate, that gives results in particle formation. The smashes of molecules are significant in slowing and preventing the settling of finer particles, mostly at a higher temperature for juice storage.

2.4.8 Alkalinity

When citrus, including oranges, lemons, limes, and tangerines, enter our body after broken down and metabolized then it develops an alkaline effect in our body. The best example of alkalizing fruits is lemon juice availability. After the lemon juice, however, other alkalinized juices are mango, guavas, nectarines, and oranges. Beets and apple juices are moderately alkaline.

2.4.8.1 Procedure

- 10 ml juice sample was brought into a titration flask and examined its initial pH with a pH meter.
- If the pH of the juice sample is basic, then use phenolphthalein (2-3 drops) as an indicator in the titration flask and swirl to mix.
- If the pH of the juice sample is neutral, then use methyl orange (2-3 drops) as an indicator in the titration flask and swirl to mix.
- Filled a 50 ml burette with 0.02 N H_2SO_4 standard solution.
- Titrated the sample while swirling the flask until the solution changed from orange to pink
- Noted the volume of acid used.
- Continued the titration until the endpoint was reached and repeated this process three times for accurate results.

2.4.8.2 Formula

$$TA\frac{1mg}{L} \text{ for } CaCO3 = \frac{A \times B \times 1000}{\text{ml of sample}} \quad (1)$$

Where, A = ml of H_2SO_4 used with only methyl orange, B = normality of H_2SO_4, A = total alkalinity

2.4.9 Carbonates

The carbonation procedure was intended to remove about 40 percent of the soluble non-sugar chemicals formed by sugar beets in the diffuser, then take them out along with the sugar. Before being mixed with a calcium hydroxide slurry known as milk of lime, the juice is heated to denature the protein. The mixture is subsequently treated with carbon dioxide gas bubbles in a vessel designed specifically for the task. The amount of gas used is sufficient to precipitate the bulk of the calcium hydroxide given as calcium carbonate and lower the alkalinity of the resultant mixture to the necessary level for the precipitate, referred to as first carbonation sludge, to settle out rapidly.

3. Results and Discussion

In our research, we have carried out the quantitative analysis of different juices by evaluating their physicochemical and bacteriological assessment.

3.1 Microbiological Analysis

Bacteria are known to cause spoilage and contamination of food items that can lead to severe health problems such as food poisoning, stomach ulcers, etc. Earlier studies have shown the presence of pathogenic bacteria and carcinogenic aflatoxins in many bakery products including juices. According to the Pakistan Standards and Quality Control Authority, there are specific standards for juices provided by the agriculture and food division. The acceptable limits of microorganisms for juices provided by this authority are less than 10 CFU/ml. According to the standards, the results of our evaluation were positive for total coliform, salmonella, and shigella as no growth was observed till 72 hours, and the agars were checked after every 24 hours for any microbial growth. This means that all of the samples were tainted with coliform, salmonella, and shigella contamination. The results in Table 1 clearly show that almost all the juices have some amount of coliform in them. This is due to the reason that juices contain different kinds of preservatives and pulps in them which have an expiry period.

Table 1: Shows the results of Microbiological analysis of local Juices

Sample No.	Juices Brands	Nutrient Agar (Cfu/ml)	E.M.B (Cfu/ml)	S.S (Cfu/ml)
1	Slice Mango	80	6	4
2	Tops	90	9	3
3	Eazu Basil Seeds	85	7	6
4	Maaza	90	6	4
5	Olpers Strawberry	85	8	9
6	Oolala	90	9	6
7	SpintaFrutbox	95	8	5
8	DayfreshPista	100	9	5
9	Tops Mix fruit	90	10	7
10	Vivo Apple	85	7	9
11	Nurpur	90	8	6

Table 2: Shows the results of Microbiological analysis of International Juices

Sample No.	Juices	Nutrient Agar (Cfu/ml)	E.M.B (Cfu/ml)	S.S (Cfu/ml)
1	Red Bull	110	25	10
2	Milo	90	20	7
3	DewDrop	95	23	5
4	Rani Orange	100	22	8
5	Nestle Mango	90	20	5
6	Fresh' N Chill	95	19	8
7	Fresher	90	17	7
8	Cappy Pulpy	100	19	8
9	Mogu	100	19	8

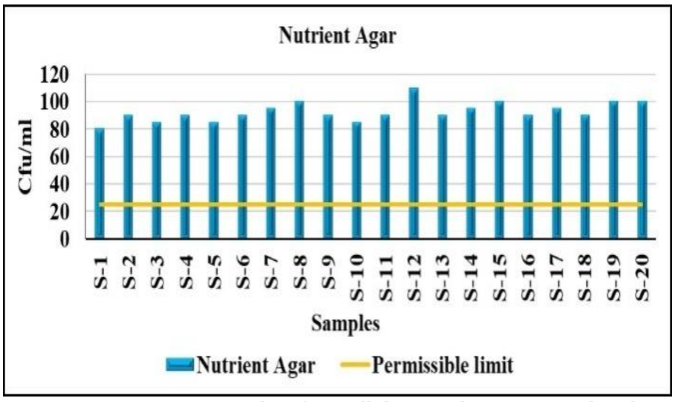

Figure 2: Represents results of N.A, all the samples are not within the permissible limit of 10CFU/ml

3.2 Physical Parameters

Turbidity is expressed as a measure of the degree to which the liquid loses its transparency due to the existence of suspended particulates. There is no permissible limit of turbidity for juices. So, fruit juices have a low pH value due to the comparatively rich in organic acid. The lower permissible limit of pH in juices is 3.5 and the upper limit should not exceed 4.7. Most of the samples are not found within the permissible limit. There is no permissible limit of EC for juices. Salts are stated as total salt in the liquid sample. There is no permissible salt content.

Table 3: Shows results of Physical Parameters

Sample No.	pH	EC (µS/cm)	Salts (mg/l)	Turbidity (ntu)
1	7.9	0.3	0	919
2	7.65	0.2	0.1	958
3	7.9	0.4	0.3	424
4	7.8	0.5	0.5	2.4
5	7.89	0.3	0.3	103
6	7.66	0.2	0.2	102
7	7.77	0.2	0.2	641
8	6.32	0.3	0.3	198
9	7.8	0.4	0.4	789
10	6	0.3	837	161
11	7.87	0.6	1.74	324
12	3.5	1.7	2.02	3.15
13	6.73	9.8	724	75
14	7.7	155	1.6	389
15	7.12	3.12	782	521
16	4.02	3.4	427	57
17	4.51	2.83	1.03	26.17
18	6.2	5.5	683	486
19	4.5	6.4	6.4	696
20	5.5	3.2	202	164

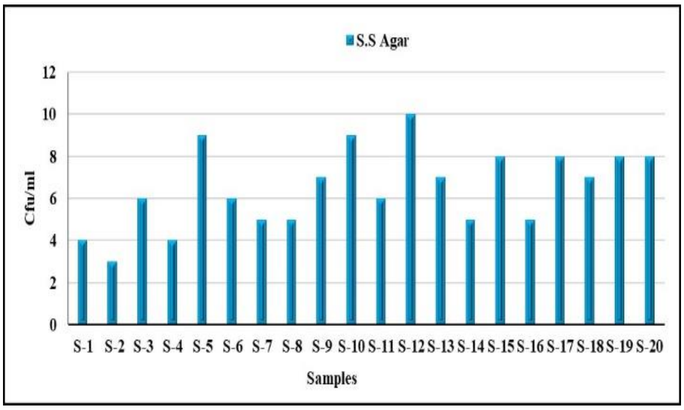

Figure 3: Represents results of S.S Agar Coliform is found in all the samples of Juice

3.3 Chemical Parameters

- Chemical parameters consist of the following:
- Carbonates
- Alkalinity

3.3.1 Carbonates

Carbonates in juices are present due to the presence of carbohydrates. Most fruit juice is loaded with sugar, but lemon and lime juices are low in carbohydrates and can help flavor other beverages. Carbohydrates are the total amount of sugars, fiber, fats, proteins, and vitamins in juices.

3.3.2 Alkalinity

Alkalinity mg/l = N*V*1000/Sample Volume,

Where N=Normality of Acid & V=Volume of Acid.

After the analysis, the results show that all the samples were found accurately detailed in Table 5.

Table 4: Shows the results of Carbonates

S. No.	$NaHCO_3$	Na_2CO_3	HCO_3	CO_3
1	635.04	801.36	461.16	453.6
2	588	742	427	420
3	618.24	780.16	448.96	441.6
4	621.6	784.4	451.4	444
5	613.2	773.8	445.3	438
6	588	742	427	420
7	613.2	773.8	445.3	438
8	649.32	819.38	471.53	463.8
9	56.78	71.65	41.23	40.56
10	722.4	911.6	524.6	516
11	649.32	819.38	471.53	463.8
12	551.04	695.36	400.16	393.6
13	668.64	843.76	485.56	477.6
14	806.4	1017.6	585.6	576
15	588	742	427	420
16	609.84	769.56	442.86	435.6
17	716.52	904.18	520.33	511.8
18	75.6	95.4	54.9	54
19	621.6	784.4	451.4	444
20	674.52	851.18	489.83	481.8

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Table 5: Shows the results of Alkalinity		
Sample No.	**Juices**	**Alkalinity (mg/l)**
1	Slice Mango	32
2	Tops	22
3	Eazu Basil Seeds	100
4	Maaza	1
5	Alpers Strawberry	57.2
6	Oolala	130.6
7	SpintaFrutbox	24
8	Day fresh Pista	50
9	Tops Mix fruit	172
10	Vivo Apple	32
11	Nurpur	32
12	Red Bull	110
13	Milo	89.2
14	Dewdrop	28
15	Rani Orange	146
16	Nestle Mango	68
17	Fresh' N Chill	28
18	Fresher	20.6
19	Cappy Pulpy	21.2
20	MoguMogu	20

4. Conclusions

In this study, collected juice samples from malls and roadside shops were analyzed for physical parameters (ph., temperature, EC, turbidity, salt), chemical parameters (total alkalinity and carbonates), and microbiological parameters (total coliforms bacteria, gram staining). From the above-mentioned results, it is concluded that the bacteriological results have not been marked satisfied due to the presence of coliform, carbohydrates and some energy drinks also have Pulps and Raw material. Results of Physical and Chemical parameters of juices show salts, pH, EC, Carbonates, and Alkalinity. There is no permissible limit of physical and chemical parameters given by PQSCA.

Recommendations

- This study highly recommends that read the ingredients of juices before drinking.
- Avoid the consumption of energy drinks on daily basis.
- Government should regulate laws regarding juice manufacturers.
- Avoid carbonated drinks

Declaration

In relation to the research, writing, and/or publishing of this paper, the author(s) disclosed no possible conflicts of interest. The author(s) also declared that this article is original, was prepared following international publication and research ethics, and ethical committee permission or any special permission is not required

Author Contributions

All authors significantly contribute to this study.

References

Agu, H. N., 2014. Physico-chemical, sensory and microbiological assessments of wheat-based biscuit improved with benniseed and unripe plantain. Food Science and Nutrition, 2 (5), Pp. 464-469.

AOAC. 1995. Official methods of analysis: an official method for fat extraction. Method no. 920.85. Association of official analytical chemists. Washington DC.

Ashurst, P., Hargitt, R., Palmer, F., 2017. Soft drink and fruit juices problems solved. Woodhead Publishing, Pp. 232.

Cerny, G., Hennlich, w., Poralla, K., 1984. Spoilage of fruit juice by bacilli: isolation and characterization of the spoiling microorganisms. Zeitscrift fur Lebensmittel-untersuching und forsching, 179 (3), Pp. 224-227.

Franke, A., Custer, L., Arakaki, C., Murphy, S.P., 2005. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. Journal of food composition and analysis, Pp. 1-35.

Giwa, O.E., and Tasleem, A.A., 2012. Microbial physical, and sensory attributes of cookies produced from wheat flour fortified with termitomyces robustus and spiced with (Allium Sativum). Journal of Pharmaceutical and Biomedical Sciences, 18 (02).

Kaddumukasa, P.P., Imathiu, S.M., Mathara, J.M., Nakavuma, J.L., 2019. Bacterial contamination of selected fruits, fresh juice contact surfaces, and Processor's Hands. A potential risk for consumers' health in Uganda. Journal of Food Sciences and nutrition research, 2, Pp. 199-213.

Yusufu, P.A., Netala, J., Opega, J.L., 2016. Chemical sensory, and microbiological properties of cookies produced from maize, African yarn bean, and plantain composite flour. Indian J. Nutrit., 3 (1), Pp. 122.