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Evaluation of Microbiological Contamination in Fruit Juices and Sensitivity of Isolates to Radiation

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Abstract

Microbiological analysis of fruit juices of four companies collected from retail businesses of four different areas of Dhaka and its surrounding area was conducted. The purpose of the survey was to improve the knowledge and understanding of the microbiological risk of locally available packed fruit juices. All samples were analysed for total viable bacterial count (TVBC), total *Staphylococcus* count (TSC), total coliform count (TCC), total faecal coliform count (TFCC), total Aeromonas count (TAC), and total fungal count (TFC). Among the juice samples (JP, JM, JA and JS), TVBC, TSC, TCC and TFC ranged between 3.5×10^4 to 2.0×10^2 cfu/ml, 2.5×10^3 to 1.3×10^2 cfu/ml, 1.9×10^2 to 1.0×10^2 cfu/ml and 2.0×10^2 to 0.5×10^2 cfu/ml respectively. Isolated microorganisms were then further identified. Among the sixteen juice samples two were found unsatisfactory and rest 14 were moderately satisfactory according to the microbiological point of view. Radiation effect on isolated microorganisms indicated that 7 kGy was effective to eliminate both *Staphylococcus aureus* and *Micrococcus luteus*; 3 kGy, 2 kGy and 1 kGy for *Escherichia coli*, *Enterterobacter aerogenes* and *Lactobacillus fermentum* and 9 kGy for *Streptococcus lactis, Alcaligenes faecalis* and *Acinetobacter colcoaceticos*. *Bacillus sereus* survived even at 10 kGy. At 3 kGy gamma radiation dose *Aspergillus niger*, *Penecillum* spp. and *Fusarium* spp. were completely eliminated.

Keywords

Microbiological Contamination, Fruit Juice, Preservation, Radiation

Sensitivity

1. Introduction

Fruit juices contain a complex mixture of nutrients which are beneficial to the maintenance of good health and have intrinsic disease risk reduction properties. In addition to the major nutrients (e.g. vitamins, minerals) inherent in the fruit itself, juices also contain phytochemicals (often referred to as phytonutrients) derived from the plant. Phytochemicals are thought to act as antioxidants and antibacterial/antiviral agents. They also have the properties to stimulate the immune system and positively effect on hormones. Considering the importance of fruit juice governments throughout the world advocate the inclusion of fruit juices in a healthy diet. A juice, that is 100% derived from its parent fruit or fruits, is almost universally regarded as a healthy and nutritious part of a human diet [1]. Besides, fruit juices are delicious and have a universal appeal especially to children and young people. Although in developed countries fruit juices commonly form part of the breakfast and are produced in very large quantities, they do not form the normal diet in our country and the fruit industry is in its infancy in Bangladesh.

These useful food items can turn into a disease-causing source if contaminated with pathogens. Juices can be contaminated in any step of their production from fruit processing to juice packing. Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts, and splits. This damage can occur during maturation or during harvesting and processing [2]. A pathogen that has become internalised within a fruit or vegetable must be able to survive in the product until it reaches the consumer in order to become a public health hazard. Most fruit juice is sufficiently acidic to inhibit the growth of pathogenic organisms. However, studies conducted on the internalisation, survival, or growth of microorganisms in fruits and juices have shown that a number of pathogenic organisms can be present and survive in a wide range of fruit and vegetables. Several studies have demonstrated the survival of microorganisms, including human pathogens, in various juices. *E. coli* O157:H7 has been found to survive in apple juice for up to 24 days at 4˚C and orange juice for 24 days at refrigeration temperatures with very little decrease in numbers [3]. Although it has been shown that pathogens can survive in orange juice, *Salmonella* and *Listeria* do not grow when the pH is below 4.4 [4] [5].

There have been documented outbreaks of illness in humans associated with the consumption of unpasteurised fruit and vegetable juices. Pathogens responsible for these outbreaks include *Salmonella* and verotoxin producing *E. coli* [6] [7]. Unpasteurised fresh orange juice contaminated with *Salmonella* was linked to an outbreak in a Florida Theme Park, USA. More than 60 visitors were affected [8]. In Australia, 427 confirmed cases of salmonellosis were reported in 1999 after the consumption of unpasteurised orange juice [9]. A total of 48 cases of *E. coli* O157

were reported after drinking unpasteurised apple juice in Washington DC in 1996 [10]. *L. monocytogenes* has also been identified as a pathogen that is of concern in relation to these products as the bacteria are present on the surfaces of raw fruits and vegetables [11].

Although a lot of criteria are involved to standardize the quality of fruit juices, and even sometimes question arises about the ingredients of juices—whether they are really fruit juices or only mixture of chemicals, our aim was to analyse the microbial quality of collected processed fruit juices from different retailers.

2. Materials and Methods

2.1. Sample Preparation

Intact packages of 250 ml fruit juice samples of four renowned companies (JP, JA, JM and JS) were purchased from different bakery and confectionary stores at shahbagh, local market in front of cardiac and kidney disease hospital, sweet and confectionary stores at saver, and local departmental stores in front of Jahangirnagar University, Savar. At least three samples were collected from each representative area of each juice samples. All the samples were immediately transported to the Laboratory of Microbiology and Industrial Irradiation Division, Atomic Energy Research Establishment (AERE), Savar, and sample preparation was started within 3 h of collection of juices. The juice samples were prepared for microbiological analysis according to the procedure described by ICMSF [12].

2.2. Microbiological Analysis

Total viable bacterial count (TVBC) was done by the standard plate count method following the method described by Sharp and Lyles [13]. Nutrient agar (pH 7.0-7.4) was used to determine TVBC as well as for isolation purposes. Plates were incubated at 37˚C for 24 h and the count was expressed as colony forming unit per ml (cfu/ml).

Total viable coliform count (TCC), total viable faecal coliform count (TFC), total staphylococcal count (TSC), and total aeromonas count (TAC) were done in the same way using McConkey agar medium, mFc agar medium, *Staphylococcus* agar medium, and Starch ampicillin agar medium, respectively. All the viable counts were the average of at least three independent experiments. Bacterial isolates were then identified according to the Bergey's manual of determinative bacteriology [14], and manual for the identification of medical bacteria [15].

Malt Yeast Glucose (MYG) chloramphenicol agar was used for fungal count and their isolation. The plates were incubated at 28˚C and counts were recorded after 5 days of growth. Viable fungal counts were the average of at least three independent experiments. The fungal isolates were identified following the procedures described by Gilman and Jones [16], Raper and Fennel [17] and Koneman *et al.* [18].

2.3. Analysis of Microbial Quality

Microbiological qualities of the studied samples were assessed according to IC-

MSF [19] and the ready-to-eat microbiological guidelines set by Food Standards Australia New Zealand [20].

2.4. Gamma Radiation Sensitivity Study

Isolated bacteria were grown to stationary phase in nutrient broth for 16 h at 37˚C. Bacterial suspensions were prepared to give about 10^8 cfu/ml using the turbidity standard of McFarland (0.5 standard). Fungal isolates were grown to stationary phase in potato dextrose broth medium for 72 h at 28˚C. Each microbial suspension of 3 ml was distributed in separate radiation tubes and subjected to 0 (control sample), 1.0, 1.5, 2.0, 3.0, 5.0, 7.0, 9.0 and 10.0 kGy at a dose rate of 12.5 kGy/h from Co^{60} gamma radiation source (50,000 Ci) at the Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh. Recovery was made by growing the cells on nutrient agar plates and potato dextrose agar plates for bacteria and fungi respectively. The specific doses (1 - 10 kGy) were selected based on prior studies on food irradiation and regulatory recommendations by FAO, IAEA, and WHO [21]-[23]. Surviving microbial counts were expressed as cfu/ml from an average value of three independent experiments.

3. Results and Discussion

Figures 1(a)-(d) show the microbiological status of the studied fruit juice samples. The TVBC, TSC, TCC and TFC ranged from 3.5×10^4 cfu/ml to 1.8×10^3 cfu/ml, 2.1×10^3 cfu/ml to 1.3×10^2 cfu/ml, 1.7×10^2 cfu/ml to 1.0×10^2 cfu/ml and 2.0×10^2 cfu/ml to 1.2×10^2 cfu/ml, respectively, in JP juices (**Figure 1(a)**); 4.9×10^3 cfu/ml to 2.5×10^3 cfu/ml, 1.9×10^3 cfu/ml to 2.0×10^2 cfu/ml, 1.9×10^2 cfu/ml to 1.0×10^2 cfu/ml and 1.3×10^2 cfu/ml to 1.0×10^2 cfu/ml, respectively, in JM juices (**Figure 1(b)**); 3.2×10^4 cfu/ml to 3.1×10^3 cfu/ml, 2.5×10^3 cfu/ml to 1.8×10^2 cfu/ml, 1.8×10^2 cfu/ml to 1.2×10^2 cfu/ml and 1.8×10^2 cfu/ml to 1.0×10^2 cfu/ml, respectively, in JA juices (**Figure 1(c)**); and finally, 3.3×10^3 cfu/ml to 2.0×10^3 cfu/ml, 1.3×10^3 cfu/ml to 3.8×10^2 cfu/ml, 1.7×10^2 cfu/ml to 1.0×10^2 cfu/ml and 1.2×10^2 cfu/ml to 0.5×10^2 cfu/ml, respectively, in JS juices (**Figure 1(d)**). Variability in microbial counts among different juice samples can be attributed to differences in processing hygiene, storage conditions, raw material quality, and potential post-processing contamination [24]-[26]. TFCC and TAC were found nil in all fruit juice samples.

A total of 46 bacteria were isolated from sixteen fruit juice samples among which 10 (21.74%) were *Staphylococcus aureus*, 8 (17.39%) were *Escherichia coli*, 4 (8.70%) were *Bacillus cereus*, 6 (13.04%) were *Micrococcus luteus*, 4 (8.70%) were *Streptococcus lactis*, 4 (8.70%) were *Alcaligenes faecalis*, 4 (8.70%) were *Enterobacter aerogenes*, 3 (6.52%) isolates were *Acinetobacter colcoaceticos*, and 3 (6.52%) were *Lactobacillus fermentum* (**Figure 2**). The presence of *B. cereus*, *S. aureus* and *E. coli* in juice samples were considered alarming since some strains of these bacterial species can cause bacterial food poisoning [27].

On the other hand, a total of 10 fungal species were isolated from different juice

samples among which 4 (40%) isolates were *Aspergillus niger*, 4 (40%) isolates were *Penicillium* spp., 2 (20%) isolates were *Fusarium* spp. (Figure 3). Presence of these fungal isolates were also considered hazardous since some species of fungus are involved in the spoilage of food which may be injurious for health [28].

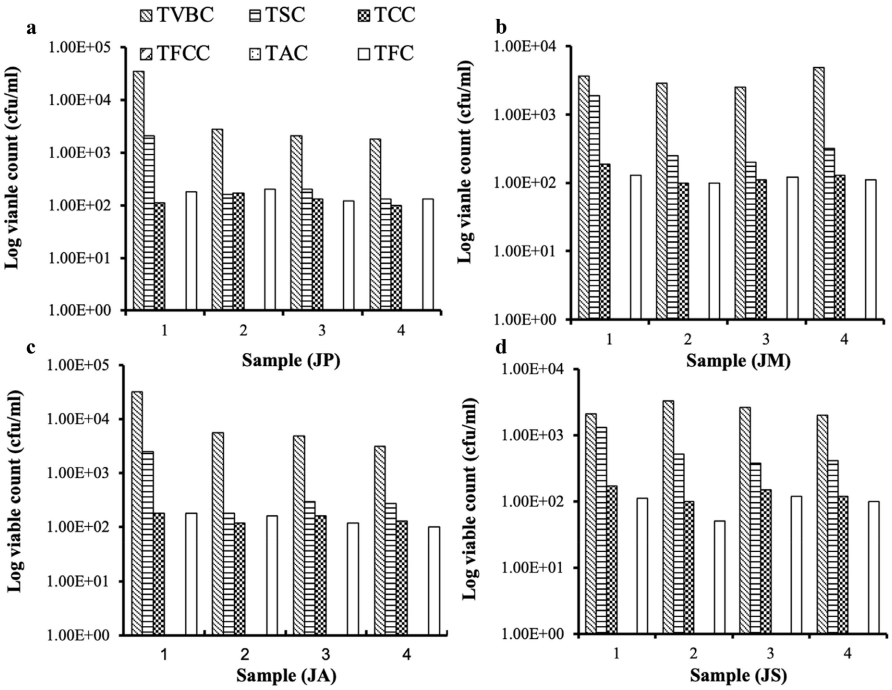

Fig. 1. Different microbiological count in the fruit juice samples

Figure 1. Different microbiological counts in the fruit juice samples.

Table 1 shows the microbiological status of the studied fruit juices on the basis of ICMSF (1978) [19] and the ready-to-eat microbiological guidelines set by Food Standards Australia New Zealand (FSANZ, 1996) [20]. The classification of samples as "unsatisfactory" or "moderately satisfactory" was based on numerical cut-offs defined by ICMSF and FSANZ, which set limits on microbial counts in ready-to-eat foods [19] [20].

Table 1. Microbiological status of the processed fruit juices.

	Area	TVBC	TCC	TFCC	TAC	TSC	TFC	Overall assessment
JP	1	M	M	S	S	US	S	US
	2	S	M	S	S	M	S	M
	3	S	M	S	S	M	S	M
	4	S	M	S	S	M	S	M
JM	1	S	M	S	S	US	S	US
	2	S	S	S	S	M	S	M
	3	S	S	S	S	M	S	M
	4	S	M	S	S	M	S	M

Continued

JA	1	M	M	S	S	M	S	M
	2	S	M	S	S	M	S	M
	3	S	M	S	S	M	S	M
	4	S	M	S	S	M	S	M
JS	1	S	M	S	S	M	S	M
	2	S	S	S	S	M	S	M
	3	S	M	S	S	M	S	M
	4	S	M	S	S	M	S	M

Note: S = Satisfactory, M = Moderately satisfactory and US = Unsatisfactory.

Among the juices one JP and one JS was unsatisfactory and the rest of the samples were moderately satisfactory. Although none of the juices were found to be potentially hazardous, the local fruit juice industries should take more care for maintaining the microbiological quality since not even a single sample could achieve the overall satisfaction level. The presence of coliform and staphylococcus mainly affected the overall assessment which indicates the probable contamination from water and air respectively. Although coliform count and fecal coliform count were satisfactory or moderately satisfactory for all samples, presence of *E. coli* in the samples was of great concern. In this study, only 8 (17.39%) isolates were *E. coli*. Since their pathogenicity was not studied, it implies a certain risk, as some serotypes of *E. coli* may cause diarrhea in infants, and their presence in large number may lead to diarrhea in adults [29] [30]. Further testing for the pathogenicity of the isolated *E. coli* strains is recommended. The same recommendation also implies to the isolated fungal strains.

Considering the presence of contaminants in the studied juice samples, it could be clearly interpreted that any preservation method should be implemented by the juice manufacturing companies before marketing their products. Since they are demanding that they are not using any preservative at least proper pasteurization can be an option for their choice. Radiation could be an alternative preservation method. Ionizing radiation has recently been widely applied in low dose (<10 kGy) level for both short term and long-term preservation of food. According to FAO, IAEA, and WHO expert committee, dose up to 10 kGy level of radiation can be safely applied for preservation of food without any nutritional or toxicological problems [31] [32]. From **Figure 4** and **Figure 5** it was evident that irradiation has effect on microorganisms associated with fruit juices. The effect of radiation on the isolated microorganisms indicated that a dose of 7 kGy was effective in eliminating *S. aureus* and *M. luteus*. *E. coli*, *E. aerogenes* and *L. fermentum* were completely eliminated at 3 kGy, 2 kGy, and 1 kGy, respectively. A radiation dose of 9 kGy was effective in eliminating *S. lactis*, *A. faecalis*, and *A. colcoaceticos*. The spore-forming *B. cereus* survived even at 10 kGy of gamma radiation. At a gamma radiation dose of 3 kGy, *A. niger*, *Penicillium* spp., and *Fusarium* spp. were completely eliminated.

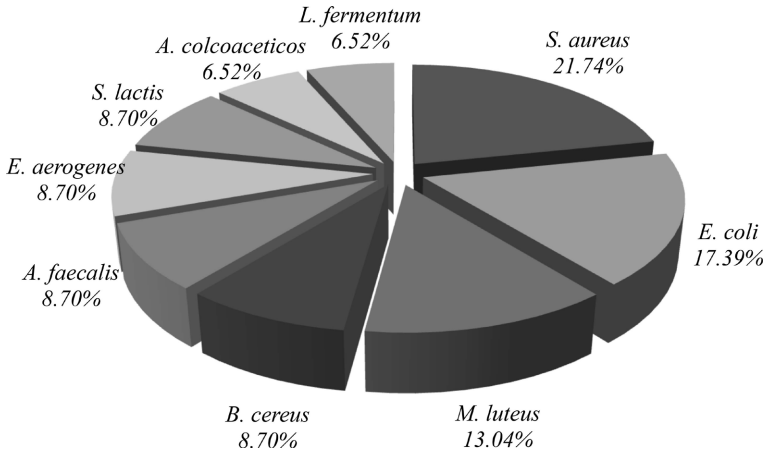

Figure 2. Distribution of bacterial isolates in fruit juices.

Among the identified bacteria, *Bacillus cereus* exhibited high resistance to gamma radiation due to its spore-forming nature, unlike *Staphylococcus aureus*, which lacks such protective structures [33]. *B. cereus* spores can survive even at 10 kGy, whereas non-spore-forming bacteria are eradicated at lower doses. In this study, at 10 kGy radiation dose, *B. cereus* count reduced from 2.3×10^8 cfu/ml to1.5×10^1 cfu/ml (*i.e.* 7 log reductions). Dymsga *et al. [34]* reported that a low dose of irradiation immediately reduces total bacteria from 10^5 to 10^3 cfu/ml in food sample, which is supported by the present findings. Stavin *et al.* [35] also reported that an irradiation dose of 1.0 to 3.0 kGy effectively reduces of bacterial load in food. Rashid *et al.* reported that bacterial counts of frozen fish samples were reduced from 10^6 to 10^4 cfu/ml at a 4.0 kGy irradiation dose [36].

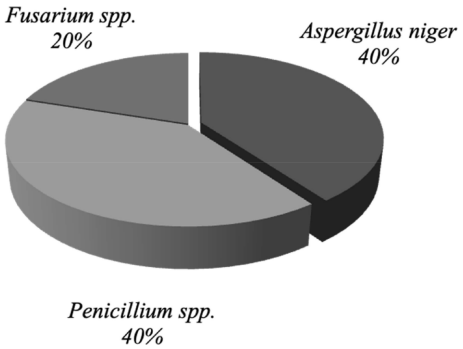

Figure 3. Distribution of fungal isolates in fruit juices.

From the radiation sensitivity study of isolated bacteria and fungi in the present investigation, it was observed that, except for the spore-forming bacterium *B. cereus*, all microorganisms were eradicated at a 10 kGy radiation dose. Therefore, a 10 kGy gamma radiation dose can be used to preserve juices if they are free from spore forming bacteria. This approach can save the consumers from ingesting harmful chemical preservatives or juices with reduced nutritional value due to extensive heat treatment. The effect of irradiation on the nutritional content,

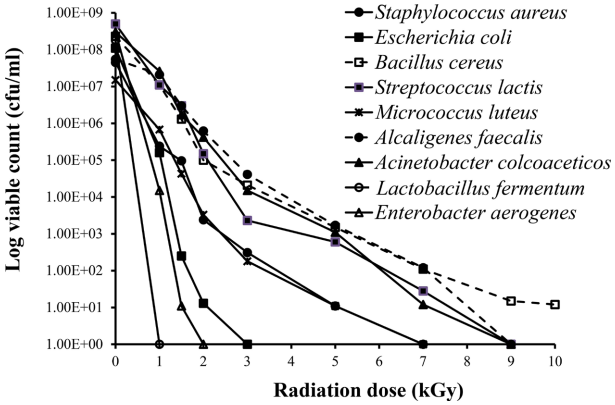

Figure 4. Radiation sensitivity of bacterial isolates present in fruit juices.

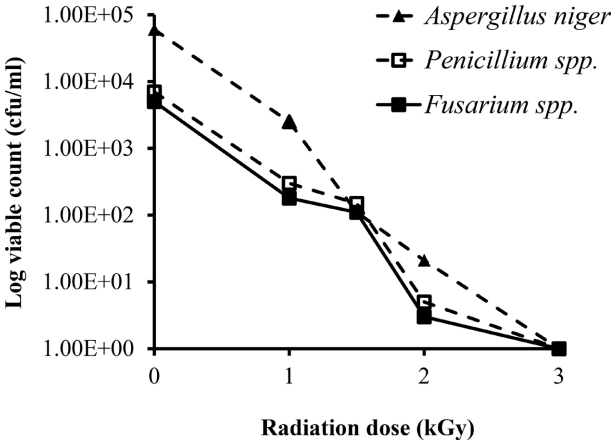

Figure 5. Radiation sensitivity of fungal isolates present in fruit juices.

sensory properties (such as taste, texture, etc.), and shelf life of the juices were not analysed in this study. However, previous studies have indicated that gamma irradiation at doses up to 10 kGy does not significantly alter the nutritional quality of foods [37] [38]. Future studies should focus on evaluating these aspects to ensure consumer acceptability.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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