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Microbiological analysis of blended RTS beverage from watermelon juice blends with bitter gourd - ginger juice during storage

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Abstract

In present investigation microbiological attributes of the blended beverage from Watermelon (*Citrulluslanatus*), Bitter Gourd (*Memordica charantia*) and ginger juice (*Zingiber officinale*) were analyzed. For the blends formulation three different proportion with one standard (v/v) as T1 (90:05:05), T2 (80:10:10), T3 (80:15:05) and T0 (100:00:00) were prepared. All samples were screened for total aerobic mesophilic bacterial counts and total yeast & moulds counts. Blends were of acidic pH ranging from 5-6.5 and decreased progressively during the storage period. Samples were found to harbor viable bacteria within range between 10^1 - 10^3 cfu/ml. Microbial activity was very low on first day of storage but it was gradually increased till 30 days of storage period to 27×10^2 . However these values were within acceptable standards for human consumption as they have not exceeded the standard values of 10^3 cfu/ml. This indicated that the pasteurization (80°C for 3 min.) of the samples was efficient and the product was safe for consumption. However the shelf life of prepared beverage was established within 30 days.

Keywords: Watermelon, Bitter Gourd, Ginger, Total Bacterial Count and Yeast & moulds

Introduction

In developing countries like India, fruits and vegetables juices sold by small vendors are widely consumed by human population. This helps to provide a readily available and affordable source of nutrition to human population including the urban and rural poor peoples. Because of fresh flavour, peoples prefer to consume unpasteurized juices and therefore in present time unpasteurized juices demand is increasing continuously. They are simply prepared by extraction by mechanical pressing. The final product is an unfermented, clouded, untreated juice ready to serve for consumption.

The major issue in these unpasteurized juices is pathogenic contamination. This contamination can enter into the fruits and vegetables through damaged surface, such as punctures, wounds, cuts and splits that occur during growing or harvesting. Contamination from raw materials and equipments, additional processing conditions, improper handling, prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables [1-3].

In recent years, consumers' awareness towards the correlation between food and health has led to an explosion of interest in "healthy foods"; this phenomenon could be partly attributed to the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for an improved quality of their later years [4]. The beverages play a very important role in the prevention of various diseases and good health. It was observed that beverages can reduce increasing burden on health care system with the help of a continuous preventive mechanism [5]. Beverages are very excellent medium for the supplementation of nutraceutical components for enrichment [6] such as soluble fiber or herbal extract [7]. The new formulations of beverages are rapidly changing.

Apart from chemical and enzymatic changes, the main problem associated with juice consumption is safety which is very serious problem. Fruit juice has increasingly been the source of serious food poisoning outbreak and fatalities. Unpasteurized juice has been implicated in outbreaks due to spp. of Salmonella; Escherichia coli, Clostridium and Cryptosporidium [8-10].

In a processed fruit juice, bacteria are the most diversified micro-organisms which can cause its spoilage. Lactic acid bacteria *Acetobacter* and *Acetomonas* found on fruit surfaces comprise

the most frequent spoilers of fruit juice because they exist on the surface of plant and fruits growing at the expense of secreted plant materials [11]. *Enterobacter spp* are commonly found on visually all types of unsound fruits in wash water. The presence of *coliform* mostly of the *E. aerogenes* type in both fresh and frozen fruit juices has been recognized to their being natural flora of fruits which may be introduced into the fruit juice if improperly processed [11]. Most fruit juices have acidic pH and have sufficient sugar, which can provide favorable condition for the growth of yeast [12]. Mould are generally considered to be the least important group of microorganisms causing spoilage in fruit juice because of their limitation, inability to grow in the absence of air [13], with the exception of few mould such as *Penicillium* and *Aspergillus* spore forming [9].

Deficiency in food safety does not only adversely impact the health of consumers but can also ruin the reputation and financial health of the offending food company. In view of the threat posed by the bacterial pathogens in juices and the flourishing demands for such juices, the present work was undertaken to assess the microbiological quality of prepared blended juices from watermelon, bitter gourd and ginger juice from outlets of Greater Noida, India.

Materials and Methods

Sampling and initial processing of samples

Freshly harvested, fully ripened watermelon and bitter Guard and Ginger fruits used for the preparation were collected from a local fruit market in Greater Noida, Uttar Pradesh India and were brought to the Food Process and Technology laboratory, GBU, Greater Noida, India. The fruits were washed thoroughly in running water. The seeds from peeled watermelon were removed manually. The collected fruits were grinded in separate and the extraction of juice was done by using an electric juicer. The extracted juices were filtered through muslin cloth. Bitter Guard and Ginger were peeled with the help of stainless steel knife, they were sliced and grounded with addition of distilled water 1:1 (v/w) and filtered through muslin cloth to have fresh juice and then stored at refrigerated temperature for analysis.

For this study one standard with 100% watermelon and three blends sets of watermelon, Bitter Guard and Ginger juice were prepared having different ratio *e.i.* 90:05:05 (T1), 80:10:10 (T2), and 80:05:15 (T3) respectively. All the samples were pasteurized at 80°C for 3 min. [14] and stored at refrigerated temperature for 30 days.

Determination of pH

The pH of the all blended juice samples was determined using pH meter using standard methods as recorded in AOAC, 2000). Experiment was carried out in triplicate at every 10 days interval up to 30 days and the mean values of the results were reported.

Isolation and estimation of microorganism from blended juice samples

Sample processing

All the prepared blended juice samples were serially diluted. Serial dilution was done in saline solution. For serial dilution 1.0mL of juice sample was diluted with 9 mL of prepared saline solution and then sample was filtered through sterile Whatman No.1 filter paper to remove solid particles. 1.0 mL of filtrate was used for inoculation in microbial detection.

Microbiological Analysis

In microbiological analysis enumeration of heterotrophic bacteria heterotrophic *e.i.* *Staphylococcus aureus*, *Salmonella*, *Shigella* and most probable number (MPN) of total *coliforms* and yeast & moulds following the standard procedure were analyzed. Appropriate dilutions were then enumerated for Total aerobic plate counts using Nutrient Agar, Coliforms using Violet Red Bile Agar, Mc Conkey Agar. Xylose. For enumeration of *Salmonella* & *Shigella*, Lysine Deoxycholate Agar was used [15-16]. Potato Dextrose Agar (PDA) was used for plate counts of yeasts and moulds [17]. All the selective media were obtained from Himedia Laboratories Ltd, Mumbai, India. Isolation was done by using pour plate method and serial dilution technique. For bacterial enumeration colony forming units (CFU) was used. For computation, average number per plate was divided by juice sample volume used in inoculation and it is expressed as CFU/100mL. Media was poured into the samples aseptically in laminar air flow using the pour plate method. The mixtures were allowed to solidify the plate was inverted and incubated at 37°C for 48 h. Colonies was counted and recorded as colony units 1 ml [18].

Statistical analysis

All the data were statistically analyzed by using one way analysis of variance (ANOVA) and analysis were carried using Microsoft Excel data analysis.

Results and Discussion

This study was executed to evaluate the quality of prepared blended juices from watermelon, bitter gourd and ginger juices by studying their physio-chemical parameters and microbiological parameters.

Microbiological analysis

The prepared blended samples were examined for their pH. During the storage period of 30 days minimum pH of the juice sample was recorded 4.51. This may be due to the fermentation process which generally took place during storage period and presence of lactic acid reducing micro-organism in the juices. The pH range of most beverages or juice products is in the ranges between 3.5 and 5.5 [19]. This decrease in the pH of beverage was due to the increment in titrable acidity which affects the organoleptic quality of juice and juice product as observed by [20].

Total Heterotrophic Bacterial Count

Total heterotrophic bacteria count in prepared blended juices for sample T0, T1, T2 and T3 was 15×10^2 , 16×10^2 , 18×10^2 and 17×10^2 cfu/ml. The bacterial count was low on first day. All the results of the bacterial counts from all the blended samples juice analyzed were within the acceptable limit (Table 1). According to the International Commission on Microbiological Specification of Foods, the acceptable limit of mesophilic aerobic bacteria in food products should not exceed a maximum of 10^3 cfu/ml. It was also found the load of viable bacteria in processed juice samples within the standard limit in the average of 10^3 cfu/ml [21]. This value steadily increased till 30 days of storage. These values were within the safe limit for juices, as they have not exceeded the standard values of 1.0×10^{10} cfu/ml [22].

Table 1: Number of total bacterial colonies per 1 ml of the sample during storage period

Treatment	0 days	10 days	20 days	30 days
T0	15	17	18	22
T1	16	19	20	23
T2	18	22	23	27
T3	17	19	22	25
Overall Mean	16.5	19.25	20.75	24.25
The <i>f</i> -ratio value is 10.59788. The <i>p</i> -value is .001086. The result is significant at <i>p</i> < .05.				
S. Ed (±)	0.645	1.030	1.108	1.108
Std. Dev.	1.291	2.0616	2.2174	2.2174

Total Yeast and Mould Count

After first day of inoculation the total yeast and moulds count in prepared blended watermelon juice, bitter gourd and ginger juice for sample T0, T1, T2 and T3 was 6×10^4 , 9×10^4 , 7×10^4 and 6×10^4 cfu/ml with the 10^{-4} dilution (Table 2). The bacterial count was low on first day. But on storage for 30 days there was slight growth in the yeast and mould growth. All the results of the yeast and mould counts from all the blended juice samples were within the acceptable and safe limit.

Table 2: Total number of yeast and moulds count per 1 ml of the sample during storage period

Treatment	0 days	10 days	20 days	30 days
T0	6	9	12	15
T1	9	11	14	18
T2	7	10	12	16
T3	6	8	10	13
Overall Mean	7	9.5	12	15.5
The <i>f</i> -ratio value is 19.75. The <i>p</i> -value is .000062. The result is significant at <i>p</i> < .05.				
S. Ed (±)	0.707	0.645	0.816	1.040
Std. Dev.	1.4142	1.291	1.633	2.0817

Total and Faecal Coliforms, Salmonella, Shigella and Vibrio Counts

No coliform bacteria were observed in all prepared pasteurized blended juice samples. None of the XLDA plates showed any black colonies of *Salmonella* or pale pink colonies of *Shigella*.

Conclusion

This study exhibited the microbiological status of prepared blended juices from watermelon, bitter gourd and ginger juices to ensure food safety for a specific control over public health risk. The average counts for bacteria of the prepared blended beverage samples examined were below the maximum allowable limit in foods to be marketed for consumption (10^3 cfu/ml). In microbiological analysis the microbial values were within the safe limit, as their numbers had not exceeded the standard values of 10^3 cfu/ml, so the developed juice beverage was microbiologically safe. Thus, blend can be recommended for production at commercial level to make nutritious and healthy RTS.

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