Microbiological Quality assessment of Hemidesmus syrup based Pomegranate Ready to serve (RTS) beverage.

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Abstract - The aim of the present microbiological study was to assess the microbial quality of Hemidesmus syrup based Pomegranate ready to serve (RTS) beverage. The blended Ready to serve beverage samples with different proportions of Hemidesmus Indicus syrup and Pomegranate fruit juice treated with various processing methods were tested for their microbial quality. Microbial quality was determined in terms of Total plate count, yeast & molds and coliform count. The total plate count, yeast and mold count were not significant upto 90th day after that it was observed <2 Cfu. The coliform count was not observed throughout the study period, indicating that the beverage shelf stability upto 3 months. Microbiological changes were observed with the progressive storage period both in carbonated and non-carbonated at ambient temperatures. The carbonated and non-carbonated samples stored at refrigeration temperature were not observed any microbiological changes.

Index Terms: Beverage, Hemidesmus indicus, Pomegranate, Microbiology, Quality assessment

1 INTRODUCTION

Beverages play an important role in maintaining body balance by preventing dehydration. Beverages are not usually consumed for their food value, but many, particularly the fruit drinks, contain quite a high percentage of sugar and therefore add to the energy content of the diet. Additionally fruit juices provide a supply of vitamins and minerals.

According to FPO (1955) a Ready to Serve (RTS) is type of fruit beverage which contains at least 10 per cent fruit juice, 10 per cent TSS and desirable amount of acidity. However, recently FSSAI in 2016 defined carbonated beverage with fruit juice as the type of beverage which contains quantity of fruit juice below 10 per cent, but not less than 5.9 per cent (2.5 per cent. in case of lime or lemon), and the requirement of TSS (Total Soluble Solids) shall not apply and the quantity of fruit juice shall be declared on the label.

Hemidesmus indicus root is said to be tonic and alterative. The native healers in India are said to use it in nephritic complaints, syphilis and in the sore mouth of children (Joseph et al., 1918). It promotes health and energy and always cures all kinds of diseases caused by vitiated blood (Pioneer herbs, 2005).The plant is said to be alterative, depurative, diaphoretic, tonic, used in autoimmune disease, rheumatoid arthritis, chronic skin disorders, asthma, bronchitis, gonorrheal neuralgia, syphilis, venereal diseases, nephritic complaints, scrofula, ulcers etc.(Global herbal,2005).

The health benefits of consuming Pomegranate fruit have been attributed to the exceptionally high antioxidant capacity which is strongly linked to the high concentration and unique composition of fruit phenolic compounds (Gil et al., 2000; Fischer et al., 2011). Pomegranates are high in polyphenols. The most abundant

polyphenols in pomegranates are hydrolysable tannins, particularly punicalagins to be the superior antioxidant responsible for the free-radical scavenging ability of pomegranate juice.

In India, traditional cuisine includes drinks, which were developed primarily to provide aesthetic appeal, though they also contain certain components having nutritional and therapeutic values. In the course of time these traditional health drinks vanished and for a long period the Indian beverage industry was dominated by aerated synthetic drinks. However, the situation has changed dramatically and witnessed a significant development in fruit based beverages.

Food quality is an important manufacturing requirement due to dietary, nutritional requirements or medical conditions of the consumers. Quality control is an important task to food and beverage to maximize shelf life and prevent spoilage and acceptability of consumers. This include external factors as appearance (size, shape, colour, gloss, and consistency), texture, and flavor; factors such as FSSAI Standards, other food safety standards and internal like chemical, physical, microbiological.

Introduction of new types of value added beverages might improve the nutritional status of the consumers and in the long run promote the socio-economic status of the country. Carbonated beverage market is dominated by carbonated beverages with synthetic colors and flavors with no fruit juice. Since, the beverage market is growing every year by more than 30%, it is necessary to develop technology for carbonation of fruit juice beverages with assured quality.

2 MATERIALS AND METHODS

2.1 Sample collection

Four different types of beverages were taken as sample for the purpose of microbiological analysis:

1) A_1B_1 : Blended + Non-carbonated RTS + Ambient storage
2) A_1B_2 : Blended + Non-carbonated RTS + Cold storage
3) A_2B_1 : Blended + Carbonated RTS + Ambient storage
4) A_2B_2 : Blended + Carbonated RTS + Cold storage

*RTS = Ready to serve beverage

3 samples of each type, total 12 samples were collected for test. The beverage samples were collected in sterile containers from S.V.University, Tirupati. The containers were kept in ice – boxes and transported to the laboratory.

2.2 Sample processing

One ml of liquid sample was taken and transferred into sterilized cotton plugged test tubes containing 9 ml of 0.1% peptone water. Then they were mixed thoroughly by shaking 20 times. This time the solution was allowed to stand for 5-10 minutes.Thus initial dilution of homogenate was prepared and from this homogenization further serial dilution were prepared.

3 MICROBIOLOGICAL STUDY

Total plate count , yeast and mold count and coliform count of fresh and stored beverage samples were determined as per the standard methods given in APHA(1992).Samples were inoculated in duplicate plates of suitable media and incubated at the recommend temperature. At the end of incubation period, the plates were counted for number of colonies.

Pour plate method was used for the determination of total viable count using total plate count agar for water; this media is unique and nonselective to count bacteria and fungi. The samples were diluted at 10 fold dilution to 10^{-6} according to American public health association sample guidelines (APHA, 1992).

Two plates from every dilution for each temperature at 36 ± 2^0c for 44±4 hrs for bacteria and 22 ± 2^0c for 68 ± 4^0c hrs foe yeast and mold were incubated.Cultural, morphological and biochemical characteristics of the isolated bacteria were studied carefully.

EMB (Eosin Methylene blue) agar was used for the isolation of E.coli as a selective medium for E.coli.

4 RESULTS AND DISCUSSION

Microbial food safety is an essential component of food quality. The microbial quality of Hemidesmus syrup based Pomegranate ready to serve (RTS) beverage processed by using different processing methods and stored at different temperatures was observed periodically. Microbiological changes were evaluated at the interval of 0, 15, 30,45,60,75 and 90 days during storage at room temperature and at refrigeration temperature.

Plate: 1 Shelf life study of the Ready to serve beverage.

The data on microbiological analysis of the Hemidesmus syrup based pomegranate ready to serve (RTS) beverage is presented in Table 1 and Table 2 .The microbial count which was taken just after one hour from preparation was found nil in all carbonated and non-carbonated beverages.

4.1 Total plate count

Table.1 Total plate count of Hemidesmus syrup based pomegranate ready to serve (RTS) beverage.

Storage Period (days)	Total plate count (Cfu/ml)			
	Treatment			
	A_1B_1	A_1B_2	A_2B_1	A_2B_2
	Mean±S.D	Mean±S.D	Mean±S.D	Mean±S.D
0	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00
45	$4.00x10^3$±0.10	0.00	0.00	0.00
60	4.70x103±0.30	3.70x103±0.20	0.00	0.00
75	5.30x103±0.10	4.00x103±0.30	3.30x103±0.10	0.00
90	5.40x103±0.10	4.20x103±0.10	3.50x103±0.10	$3.00x10^3$±0.20

Note: Treatments: A_1B_1 Blended + Non-carbonated RTS + Ambient storage

A_1B_2 Blended + Non-carbonated RTS + Cold storage

A_2B_1 Blended + Carbonated RTS + Ambient storage

A_2B_2 Blended + Carbonated RTS + Cold storage

The total plate count was nil at initial stage of storage. The effect of storage on the quality of Hemidesmus syrup based Pomegranatea ready to serve (RTS) beverage was assessed during a storage period of 90 days with an interval of 15 days.(Table.1).

Total plate counts were not observed up to 30 days of storage in all beverage samples. Total plate count in Hemidesmus syrup based Pomegranate ready to serve (RTS) beverage was found in the range of $4.00x10^3$±0.10 to 5.40x103±0.10 Cfu/ml in the sample A_1B_1 from the 45th day to 90th day.

On 60th day of storage $3.70x10^3$±0.20 Cfu/ml microbial load was recorded in A_1B_2 and gradually increased upto $4.20x10^3$±0.10 Cfu/ml on 90th day. In the sample A_2B_1 on 75thday of storage $3.30x10^3$±0.10 Cfu/ml total plate count was recorded and increased to 3.50x103±0.10 as the storage period increased up to 90th day.

The minimum total plate count ($3.00x10^3$±0.20 cfu/ml) was observed in the sample A_2B_2 after 90 days of storage.

4.2 Yeast and Mold count

Table.2 Yeast and mold counts of Hemidesmus syrup based pomegranate ready to serve (RTS) beverage.

Storage Period (days)	Yeast and mold count (Cfu/ml)			
	Treatment			
	A_1B_1	A_1B_2	A_2B_1	A_2B_2
	Mean±S.D	Mean±S.D	Mean±S.D	Mean±S.D
0	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00
75	$1.20x10^3$±0.10	0.00	$1.20x10^3$±0.10	0.00
90	$1.40x10^3$±0.10	$1.20x10^3$±0.10	$1.30x10^3$±0.10	$1.10x10^3$±0.10

Note: Treatments: A_1B_1 Blended + Non-carbonated RTS + Ambient storage

A_1B_2 Blended + Non-carbonated RTS + Cold storage

A_2B_1 Blended + Carbonated RTS + Ambient storage

A_2B_2 Blended + Carbonated RTS + Cold storage

Yeast and mold counts in Hemidesmus syrup based Pomegranate ready to serve (RTS) beverage were nil at initial days of storage. Yeast and mold counts were not recorded upto 60 days storage while after 75 days the yeast and mold count were between $1.10x10^3$±0.10 to $1.40x10^3$±0.10 cfu/ml.

4.3 Coliform count

Coliform count was found to be nil throughout the storage period at refrigeration temperature

.This indicated that the Hemidesmus syrup based Pomegranate ready to serve (RTS) beverage remained safe microbiologically throughout the storage period and no appreciable changes was observed during storage.

Thorat et.al. (2007) reported that the microbial count was nil at initial stage of storage but increased as the storage period advanced. Minimum microbial count ($6x10^3$ Cfu/ml) was recorded in carbonated Anola fruit juice based health drink at cold storage after 90 days. The data was found in conformity with the results observed in the present study.

5 CONCLUSION

The current study revealed that all the studied beverage samples had lower microbial load than the specification. From the data presented in the study, it could be concluded that the microbiological quality of most of the beverages tested were satisfactory and within acceptable permissible limits.

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