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Preparation and microbial evaluation of RTS beverage (punch) prepared with lactic acid fermented carrots and sweet lime juice

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ABSTRACT

RTS beverages were prepared using fermented carrot juice and sweet lime. Carrots were fermented with *Streptococcus lactis* and *Lactobacillus plantarum* at ambient temperatures with 2 percent bacteria and 2.5 percent salt for 24-48 hours. Juice of the fermented carrots was combined with sweet lime juice in 50:50 (V1 and V4), 75:25 (V2 and V5) and 25:75 (V3 and V6) ratios. The beverages were prepared as per FPO specifications and organoleptically evaluated. The TSS value of RTS beverages ranged between 14^0B to 21^0B. There was no microbial growth in RTS beverages prepared with fermented carrots up to 30 days of storage. The increase in microbial load after 45 days of storage was negligible and safe for consumption; however the increase was substantial in the standard.

Introduction

RTS beverages have been increasingly gaining popularity throughout the country due to their health and nutritional benefits, apart from pleasant flavour and taste. Fruit based RTS beverages are not only rich in essential minerals, vitamins and other nutritive factors but also are delicious and have a universal appeal. Punches have been prepared with different combinations of fruit juices. However, the concept of preparation of punches with vegetable and fruit juice combination is not yet known. The acid content of vegetables is low. This can be increased by fermenting the vegetables using bacteria.

It is also quite a challenge to prepare RTS beverage without the addition of chemical preservatives and coloring. Therefore preparation of RTS using fermentation as a method of preservation in which the high acid content of the carrot pulp replaces the need for additional citric acid has been studied.

The study was planned with following objectives:

- To study increase the acidity of carrots by fermentation with lactic acid bacteria.
- To formulate RTS punch using sweet lime juice and lactic acid fermented carrot.
- To analyze the shelf life of the products.

Methodology

Procurement of Raw Materials

The raw materials used in the preparation of RTS beverages were carrot and sweet lime (musambi) and other ingredients including sugar, water and salt. Two species of lactic acid producing bacteria were used for fermentation. The two bacterial species were obtained form MTCC (avoid two times species) (Microbial Type Culture Collecting Centre) Chandigarh, India. The bacterial species namely *Lactobacillus plantarum (1407)* and *Streptococcus lactis (440)* were obtained in a lyophilized form. They were subcultured in specific media and confirmed using standard microbiological and biochemical tests and used for the present study.

Activation of Bacteria

The procured cultures were first activated in MRS (De Man, Rogosa and Sharpe) broth (Plate 1). The slants, stored under refrigerated conditions (4-8^0C) were first aseptically transferred to MRS broth and incubated for 24-48 hours at 32-37^0C (Sharma, Joshi and Lal, 2008). As per the procedure followed by Ray and Bhunia (2008), controlled or pure culture fermentation was carried out in the present study by growing the microbial species associated with this fermentation in large volume in the laboratory and then added to the raw material in very high number.

Fermentation of Carrots

Carrots were washed and cleaned thoroughly by scrubbing to remove the adhering sand/mud and washed in running water and finally immersed in warm sterilized water for 15 minutes to kill the soil bacteria if any. Then the external skin was peeled and carrots were shredded and mashed to speed up the fermentation.

Most of the vegetables can be fermented naturally when kept in brine solution for sufficient time at appropriate temperature (Pederson, 1979) and (Vaughn, 1985). The mashed carrots were mixed with dry salt (2.5%), and inoculated with *Lactobacillus plantarum* and *Streptococcus lactis* separately at the rate of 2 percent ($6x10^4$cfu/ml). The fermentation was carried out in aerobic condition by covering the vessel using a muslin cloth for 24-48 hours to produce the acidity.

Preparation of RTS Beverages

Fresh sweet lime juice was prepared by cutting the sweet lime into halves and the juice was extracted using a juice extractor similar to the study by Shilpa and Rajyalakshmi (2009) on Quality and Storage of RTS Beverages from Bael and Citrus Fruit Blend. The RTS beverage was prepared by using lactic

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acid fermented carrot juice, freshly prepared sweet lime juice, water, and sugar as per FPO specifications (Gridharelal *et al*., 1998).

Plate 1
Display of Bacteria in Broth Culture

Streptococcus lactis *Lactobacillus plantarum*

- The methodology followed in Phase I is shown in Figure 1.

Carrot and sweet lime juices were mixed in three different proportions i.e. 50:50, 75:25 and 25:75 respectively (Figure 2). The sugar syrup was prepared separately up to the boiling stage, and strained through muslin cloth and added after cooling, to get desired TSS of 14^0B-20^0B (FPO specifications). The combined carrot and sweet lime juices were then mixed with the sugar syrup in the correct quantity to get the RTS beverage. Also a RTS beverage was prepared with unfermented carrot and sweet lime juice for comparison.

Figure 1
Phase-1: Fermentation of carrot

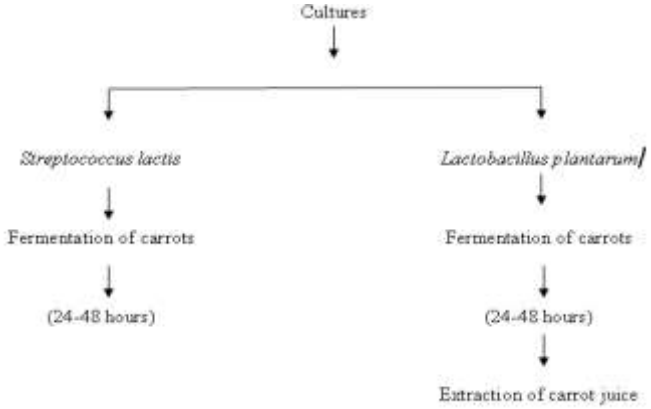
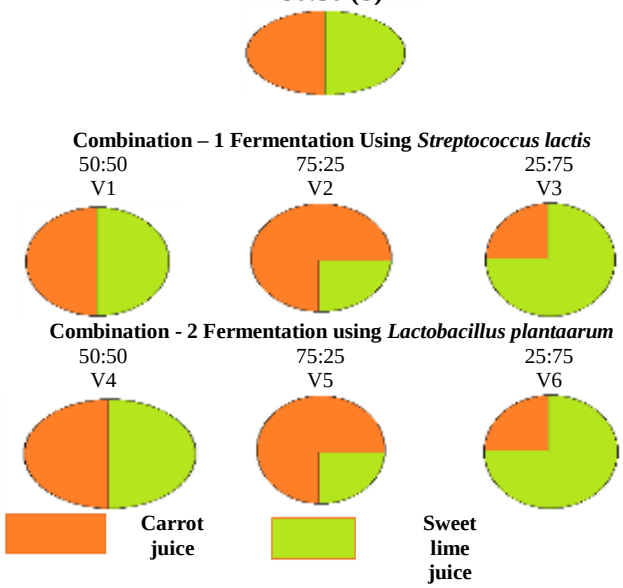


Figure 2
Formulation of RTS Beverages +Standard RTS
Unfermented carrot juice: Sweet lime juice.
50:50 (S)



The beverages were filled in pre-sterilized glass bottles of 200ml capacity (Plate 2) by leaving one inch head space and crown corked and sterilised for 30 minutes in boiling water bath followed by cooling. The bottles were stored under refrigeration conditions (Barwal *et al*., 2006; Saravanakumar and Manimegalai, 2002; and Kaushal *et al*., 2008).

Plate 2
Display or RTS Beverages

Standard V1 V2 V3 V4 V5 V6
Streptococcus lactis *Lactobacillus plantarum*

P^H

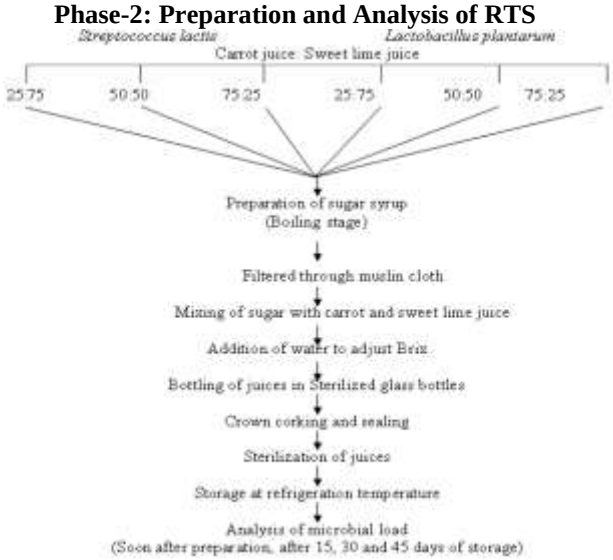
The pH of the medium has a profound effect on the heat resistance bacterial spores which becomes maximum at pH values between 6 and 7 (Ranganna, 1986). Fruit products are being effectively preserved at low pH (Sindhu *et al*., 1984).The ph was measured using a ph meter during fermentation, preparation and at different storage periods.

Microbial Analysis

Contamination of foods by mould or bacteria is common. Hence their presence in the finished product is considered unfit for consumption (Ranganna, 1986). The micro organisms in processed foods are sometimes inherent to the external environment. It is virtually impossible to process the foods to sterile products without altering the organoleptic changes in many cases.

Microbial analysis was done by total plate count (TPC). Standard plating in nutrient agar was carried out. This is called as total plate count method. . The total microbial load (TPC) of RTS beverage was determined in nutrient agar media according to the method given by Harrigan and McCance (1966), soon after preparation, after 15, 30 and 45 days of storage.

Figure 3
Phase-2: Preparation and Analysis of RTS



Results & Discussion

P^H

The acidity of the fermented carrots was checked after 24 hours at 6 hour interval.. Acidity acts as a preservative here. Harman *et al*., (2007), Singh and Dhawan (1983), Murari and

Verma (1989), Pandey and Singh (1999) reported that high acidity in guava pulp is a desirable character as it provides better storage quality. On completion of fermentation (when no further increase in acidity was observed), carrot fermentation was stopped by extracting the juice from it (Sharma and Joshi, 2007). In the present study, when the acidity of the fermented carrots, reached pH 4.5 fermentation was terminated. The juice was strained and filtered through a muslin cloth to obtain clarified, clear juice as per the procedure followed by Nakadi *et al*., (2000) and Harman and Amuth, (2007) in their study on RTS beverages and carbonated and sapota beverages respectively. The pH values of RTS Beverages are given in Table 1.

An acidic pH was maintained throughout the study period which helped in the preservation of RTS beverages but the pH values of all the RTS beverages increased during 45 days of storage.

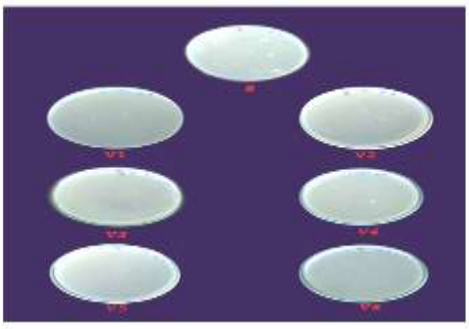
Ranote *et al*., (1992) reported a negligible change in pH of Kinnow RTS during 24 weeks of storage. Broody and McClellan (1986) have studied the effect of pH and acidity on the shelf life of inoculated apple juice and found that it was acceptable at different levels of storage. Same trend was reported by Sahni and Khurdiya (1989) in mango RTS beverage during storage. In a study conducted on pH and citric acid values of grape fruit juices, samples stored at 5^0 C for 12 weeks showed no change in the values showing that the acid environment of the juices does not change during storage (Robertson and Somaniego, 1986). However, Saravanakumar and Manimegalai (2005) have reported a decrease in pH from 6.12 to 5.70 after 90 days of storage.

Microbial Count

It is clear that there is no microbial growth in the RTS beverages prepared with both the micro organisms (Table 2 and Plate 3). The increase in microbial load after 45 days of storage was negligible and safe for consumption, as also echoed by Nagpal and Rajyalakshmi (2009), in their study on RTS beverages from bael and citrus fruit blends. Since fermented carrots were not used in the standard, there was microbial growth. This shows that the acid environment that was maintained as a result of fermentation has prevented microbial growth. It is also well known that the microbiological safety of fruit juices depends upon the formulation, raw materials, maintenance of pH and the processing conditions Contamination of raw materials and equipments, additional processing conditions, improper handling, prevalence of unhygienic conditions contribute substantially to the entry of pathogens in juices with vegetables and fruits (Oliveria et.al.2006; Nicolas *et al*., 2007). Most fruits contain bacterial counts of 1x103cfu/cm2 on their surface (Harrigan1998; Al Jeddah & Robinson 2002). Since in the present study all these safety precautions were taken care of, there was no microbial growth in the RTS beverages.

Plate 3

Microbial Load after 45 Days of Storage



Conclusion

The RTS beverages (punch) prepared with sweet lime juice and lactic acid fermented carrots had a good shelf life without the addition of artificial preservatives and acid. Thus it can be used to replace the synthetic beverages which are devoid of nutritional value.

Recommendations

- The RTS beverages of the present study can be stored for a longer storage period and shelf life studies conducted.
- Other microbes can be analyzed.
- Vegetables other than carrot can also be fermented and similar studies conducted.

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Table 1: pH Values of RTS Beverages

S. No.	Types of RTS Beverages	pH			
		Soon After Preparation	After 15 Days of Storage	After 30 Days Storage	After 45 Days of storage
1	Standard	4.0	4.24	4.35	4.46
2	V1	3.5	4.27	4.29	4.28
3	V2	3.8	4.18	4.42	4.46
4	V3	4.0	4.20	4.23	4.34
5	V4	3.8	4.20	4.16	4.18
6	V5	3.9	4.25	4.24	4.29
7	V6	4.0	4.21	4.16	4.22

Table 2 Microbial Load of RTS Beverages

Variation	Microbial Colonies (CFU /ml)			
	0^{th} Day	15^{th} Day	30^{th} Day	45^{th} Day
Standard	-	-	-	$40*10^4$/ml
V1	-	-	-	$3*10^4$/ml
V2	-	-	-	$2*10^4$/ml
V3	-	-	-	$3*10^4$/ml
V4	-	-	-	$3*10^4$/ml
V5	-	-	-	$3*10^4$/ml
V6	-	-	-	$2*10^4$/ml

CFU- Colonies Forming Unit