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Biodiversity

Screening of Cashew Apple Juice for its Potential to Promote Growth of Probiotic Bifidobacterium spp

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Abstract **Original Research Article**

The aim of the study was to assess the efficacy of cashew apple juice (CAJ) in dairy and non dairy medium to promote growth of probiotic strains (*Bifidobacterium infantis* and *Bifidobacterium lactis)* Different combinations were made using CAJ at 10%, 20%, 30%, 40% with orange juice or whey as dairy and non- dairy medium and the growth of the probiotic strains were checked individually in the different blend ratios. The probiotic growth was comparable between dairy and non dairy mediums for both *B.lactis and B.infantis.* The sensory evaluation of the blends resulted in highest sensory score at 30% (30:70 CAJ:dairy/non dairy) for both the mediums and the means for overall acceptability scores between the blends had statistically significant difference. The ideal synbiotic combination of 30: 70 for CAJ:orange juice and CAJ : whey was optimised based on sensory results and higher viable counts. The ideal synbiotic combinations with 1% innoculum of probiotic cultures individually were incubated for 4 hours. The 30:70 ratio of CAJ: orange juice and CAJ: whey showed an increase in viable count, acidity and decline in pH as the incubation time progressed. The results obtained in this study suggests that cashew apple juice as a natural prebiotic source can be recommended for further use to formulate products that promote gut health.
Keywords: Cashew apple juice. Prebiotic, Bifidobacterium sps, gut health.

INTRODUCTION

Probiotic, prebiotic and synbiotic are words of the modern era, bookmark “for life” and is in use to define bacterial association with beneficial effects on human health [1].The health benefits imparted by probiotics and prebiotics as well as synbiotics have been the subject of extensive research in the past few decades. These food supplements termed as functional foods have been demonstrated to alter, modify and reinstate the pre-existing intestinal flora and also facilitate smooth functions of the intestinal environment [2].

Diet is considered as one of the main drivers in shaping the gut microbiota. Microflora modulation can occur through food stuffs that contain sufficient level of growth supplements (prebiotics) that target the (potentially) health promoting flora. Dietary modulation studies have clearly established the abilily to modify the gut microflora [3, 4]. The combination of suitable prebiotics with probiotic/s has been found to enhance the survival and activity of the organism, both in vitro and in vivo experiments, for example a fructooligosaccharides in conjunction with a Bfidobacterium strain or lactitol in conjunction with Lactobacillus [5].

Healthiness in many countries is linked with naturalness [6]. A function that re-enforces the natural properties of a product is easier to accept than a function that is artificially implanted to the product. Therefore, adding probiotics into food products requires, in addition to technological considerations, thought concerning the beliefs consumers have about these products [7].

Consumption of appropriately selected probiotics as well as prebiotics may enhance the beneficial effect and when presented naturally the consumer beliefs. Fruit juices serves as ideal substrates for the culture of probiotics, since they already contain beneficial nutrients such as minerals, vitamins, dietary fibres, and antioxidants [8]. Consumers are increasingly aware of the health benefits that foods can provide and research in this area of using fruit juices as a delivery for probiotics is gaining a lot of interest. Cashew apple is considered an agriculture waste, and its nutritive juice can be a utilised as a suitable low-cost substrate for the delivery of probiotics This study explores the ability of

Cashew apple juice in dairy and non dairy medium as a natural substrate for the growth of *Bifidobacterium* sps.

The objective of the study is to evaluate the ability of the probiotics *Bifidobacterium lactis and Bifidobacterium infantis* to utilize Cashew apple juice (CAJ) as a prebiotic substrate in dairy and non- dairy medium individually, to explore its prebiotic potential. Further investigates the probiotic growth in the ideal synbiotic combination by determining the growth, viability and specific chemical changes during the progress of incubation.

MATERIALS AND METHODS

Procurement of materials

Cashew apples were procured and stored under refrigeration for further processing. Liquid whey was prepared using citric acid (2%) and stored for further use [9]. Fresh Oranges were procured from the local market and used to obtain orange juice required for the study.

Probiotic Strain

Proven probiotics strain of *Bifidobacterium lactis* (UBBLa-70) and *Bifidobacterium infantis* (UBBI-01) was obtained from Unique Biotech in the lyophilized form for the study.

Starter Culture

Starter culture was prepared by incubating the culture in Bifido broth overnight at 37°C under anaerobic condition to obtain the working culture. This was maintained at 5˚C and sub cultured in Bifido broth.

Preparation of fruit sample and extraction of juice /pulp

The selected fruits were washed and cleaned with potable water. The inedible parts like seed, rind, peel were removed and the juice extracted from the pulp was stored frozen for further use. The fruit juices, whey were individually heated at 85 ºC for 5 minutes and used in the study.

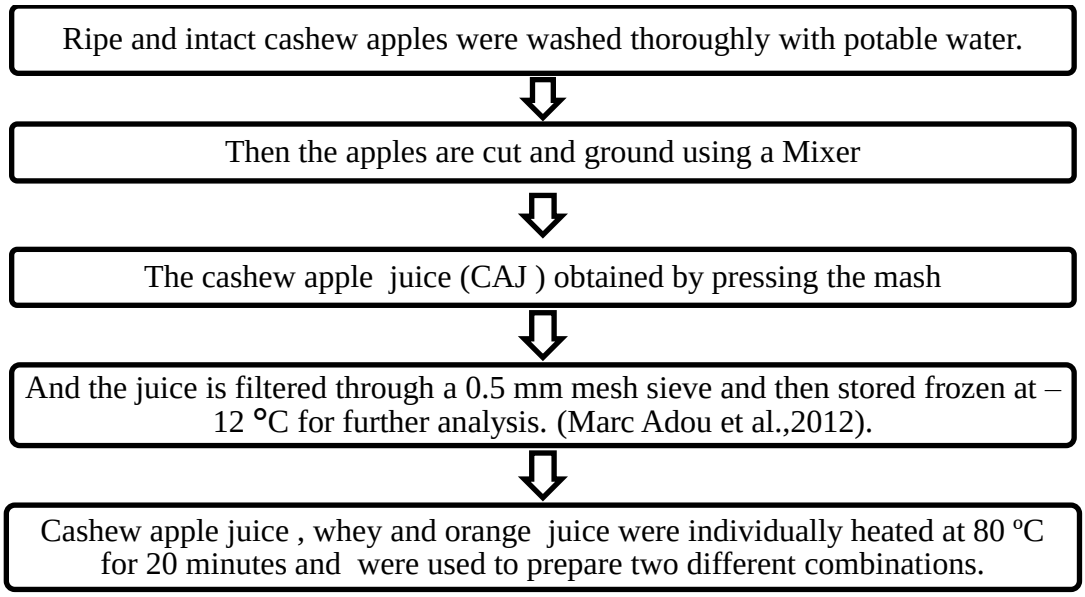

Fig-1: Flow chart for Cashew apple juice preparation

Assesing the ability of the probiotics *Bifidobacterium lactis and Bifidobacterium infantis* to utilize Cashew apple juice (CAJ) as a prebiotic substrate in dairy and non- dairy medium individually

Determining Bacterial Viable count of probiotic culture (*B.lactis & B.infantis*)

Bacterial viable count (CFU/ml) was evaluated by plate count technique in triplicates under anaerobic conditions [10].

Assessing the chosen dairy and non dairy substrates for promoting growth of the probiotics

Whey and orange juice were checked for their suitability as a potential dairy and non dairy substrate for *B.lactis* and *B.infantis* by inoculating the probiotic cultures (100µl in 10 ml of substrate), incubating for 24 hours and determining the bacterial viable counts and optical density.

Preparation of blend ratios

The CAJ based dairy blend was prepared with Cashew apple juice and Whey and non dairy blend was prepared with Cashew apple juice and orange juice in different ratios.

Table-1: Blend ratios

FRUIT: NON DAIRY MEDIUM		FRUIT: DAIRY MEDIUM	
CAJ : Orange juice		**CAJ : whey**	
Cashew apple juice (10:0)		Cashew apple juice (10:0)	
NDM1	01:09	DM1	01:09
NDM2	02:08	DM2	02:08
NDM3	03:07	DM3	03:07
NDM4	04:06	DM4	04:06

Note: 10 % of sugar was added uniformly to all the blends

Determining growth of probiotic culture (*B.lactis & B.infantis*) in the blend ratios using Turbidimetric method

Turbidimetric method is a simple, rapid method used for following growth of bacterial cultures. Bacteria in a suspension absorb and scatter the light passing through them so that a culture of 10^7 to 10^8 appears turbid to the naked eye [10]. The indirect method of determining the optical density before and after incubation was estimated and used to represent growth.

Viable count analysis to assess the ability of *Bifidobacteria lactis /Bifidobacteria infantis* to utilize different ratio of fruit: dairy and fruit: non dairy medium as a Prebiotics substrate for growth

All the above blends were used as a growth medium for the probiotic strains used in the study. Inoculation of the probiotic cultures and assessing prebiotic effect of the combinations was determined using viable counts [11]. To all the above combinations 1.0% of each culture was inoculated into the substrate and viable bacteria counts were performed in triplicates. Both cultures were assessed individually for their ability to promote growth. The blends were enumerated on selective Bifido agar and grown under anaerobic conditions in Anaerobic jars (GasPak System) for 48 hours at 37^0 C [12].

Sensory evaluation

The appropriate concentration of fruit to be used for further study was determined by blending different ratio of CAJ:Whey and CAJ: Oeange juice and the blends were subjected to sensory evaluation using five point hedonics scales to determine the best combination that would be acceptable to the consumers.

Analysis of the ideal synbiotic combination

Growth of the ideal symbiotic combination (Fruit: Dairy/Non diary medium) during 4 hours of incubation was assessed by determining optical density, viable counts and chemical changes. The ideal combination was freshly prepared and 1.0 % of each probiotic culture was inoculated for the study.

For an enumeration of bacterial population growth, viable cells are inoculated into the sample and under appropriate conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth pattern, which is constructed by plotting the increase in cell numbers verses time of incubation [9].

Estimation of Viable counts and turbidity 0 hour and 4 hours

In the direct method number of colony forming unit at various time intervals were estimated and for the indirect determination method the optical density was estimated.

Measurement of specific chemical changes produced on the medium.

The measurement of acid or any other end product is a very indirect approach to the measurement of growth of the bacterial population.Analysis like pH and TTA were measured to determine the amount of acid produced under specific conditions during a fixed period of time and is proportional to the magnitude of the bacterial population [10].

The pH was measured using the Digital instrument (pH meter).The Titrable acidity was determined by the method given by AOAC [13] and the acid produced during fermentation was expressed as % TTA. Both pH and TTA values of the formulated beverage was measured at 0, 2 and 4 hours in triplicates.

RESULTS AND DISCUSSION

The results obtained on screening the Cashew apple juice in dairy and non dairy medium for its ability to promote growth of *B.lactis* and *B.infantis* are compiled and the data assembled as tables,graphs and charts are presented .

Total viable count of the probiotic culture

The Bacterial viable count using the total plate count method was found to be 9.0492±0.0625 and 9.9242±0.0295 log cfu/ml for the probiotic culture of *B.lactis* and *B.infantis* respectively.

Whey and Orange juice as a potential dairy and non dairy substrate for *B.lactis* and *B.infantis*

The assessment of orange juice and whey was done to make ideal combinations of fruit: dairy / non dairy mediums to cater to a diverse population.

Viable plate count

Table-2: Total Viable count in dairy and non dairy medium (B.lactis and *B.infantis*)

Viable count	**Substrate Used For Probiotic Growth**			
	Whey		***Orange juice***	
	B. lactis	***B.infantis***	***B. lactis***	***B.infantis***
Initial viable count log cfu/ml	9.0492±0.0625	9.9242±0.0295	9.0492±0.0625	9.9242±0.0295
Viable count after 24 hours on incubation log cfu/ml	10.1095±0.0019	11.0767±0.0562	10.2676±0.3379	11.2669±0.0202

The viability of both the species (*B.lactis and B.infantis*) in whey and orange juice after 24 hours of incubation showed an increasing trend. Richardson *et al*. [14] pioneered work on the use of whey as a low-cost alternative medium for the propagation of lactic starter cultures and reported good results. Sheehan *et al*. [15] found that the orange juice acts as a good substrate for the growth of probiotics and *B.lactis* showed no significant decrease in viability over the four weeks of storage in OJ and numbers remained above 10^6 cfu ml.

Turbidimetric method

On using turbidimetric method there was increase in turbidity in both the strain (B.lactis and B.infantis) after 24 hours of incubation for the dairy and non dairy medium.

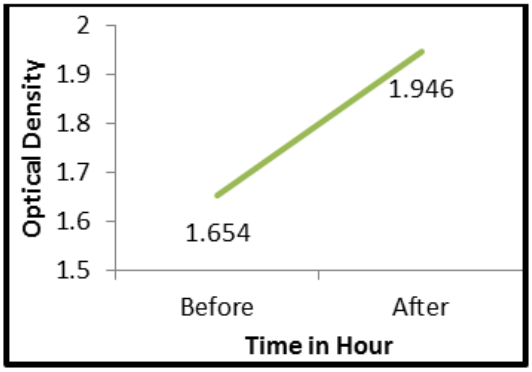

Fig-2: Change in turbidity in orange juice (*B.lactis*) in 24 hours

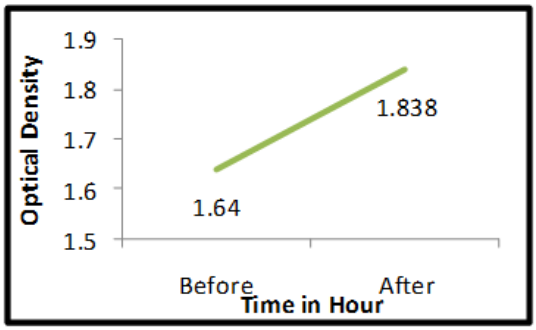

Fig-3: Change in turbidity in orange juice (*B.infantis*) in 24 hours

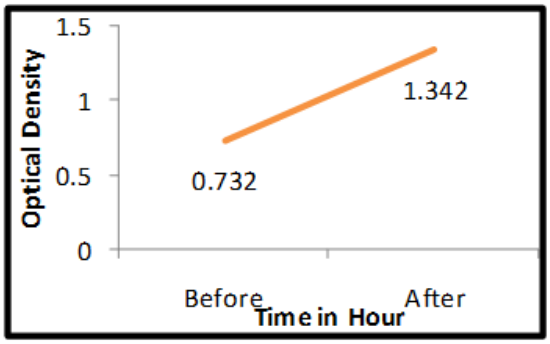

Fig-4: Change in turbidity in Whey (*B.lactis*) in 24 hours

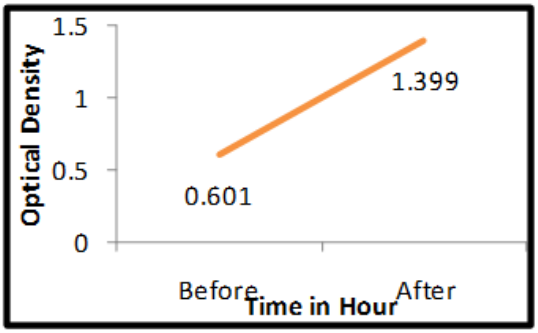

Fig-5: Change in turbidity in Whey (*B.infantis*) in 24 hours

The increase in log cfu/ml and turbidity measured after incubation reveals that both the substrates support the growth of probiotic cultures which is in concurrence with the studies reported. Hence these two substrates were used in the study to support the growth of the probiotics as dairy and non dairy medium with CAJ.

Assessing the ability of the probiotics to utilize fruit: dairy (or) non-dairy blend ratios as prebiotic sources

Viable plate count of CAJ: Orange juice and CAJ: Whey (*B.lactis and B.infantis*) after 24 hours of incubation

From the initial values of 9.0492±0.0625 and 9.9242±0.0295 log cfu/ml for *B.lactis* and *B.infantis*, for both the probitoic culture inoculated in different ratio of CAJ: Orange juice and CAJ: whey an increase in the total viable count was observed with maximum growth observed in 03:07 ratio in the dairy and non dairy medium blend.

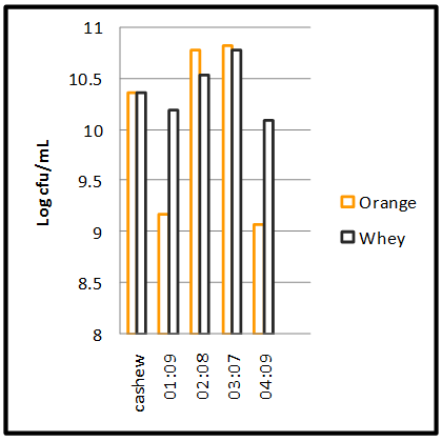

Fig-6: Viable counts in CAJ and blends of CAJ: Whey and CAJ: Orange using *B.lactis* after 24 hours of incubation

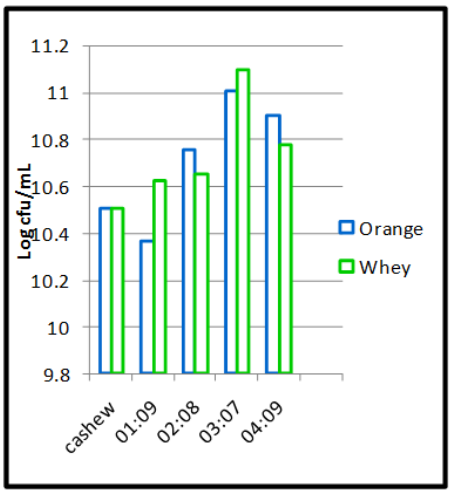

Fig-7: Viable counts in CAJ and blends of CAJ: Whey and CAJ: Orange with *B.infantis* after 24 hours of incubation

Turbidemetric method

On using turbidimetric method there was increase in turbidity in dairy and non – dairy medium after 24 hours of incubation.

Table-3: Change in turbidity of CAJ: Orange juice, CAJ: whey (*B lactis*)

Blend ratios	Before incubation	After incubation	Difference in OD values before and after incubation
Cashew(10:00)	1.608±0.044	1.919±0.0005	0.268
CAJ: ORANGE BLEND RATIOS			
01:09	1.446±0.072	1.867±0.031	0.421
02:08	1.458±0.016	1.870±0.005	0.412
03:07	1.325±0.039	1.872±0.006	0.547
04:06	1.698±0.003	1.859±0.025	0.161
CAJ: WHEY BLEND RATIOS			
01:09	1.318±0.106	1.625±.018	0.308
02:08	1.206±.016	1.656±0.075	0.450
03:07	1.242±0.118	1.796±0.010	0.554
04:06	1.495±0.012	1.697±0.008	0.203

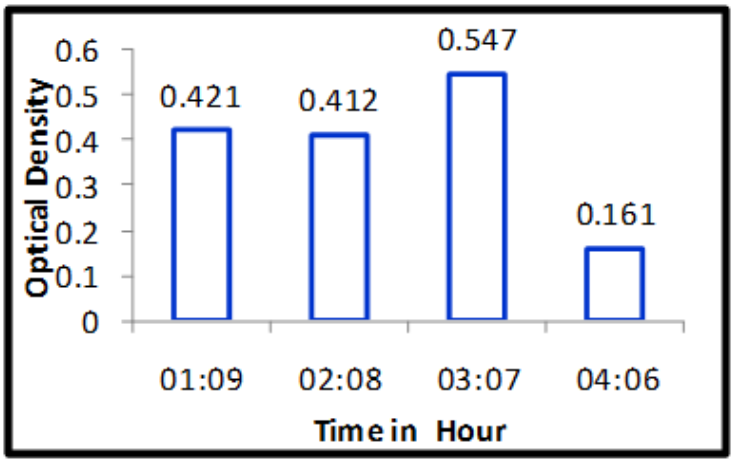

Fig-8: Difference in optical density of CAJ: Orange *B.lactis* before and after 24 h hours of incubation

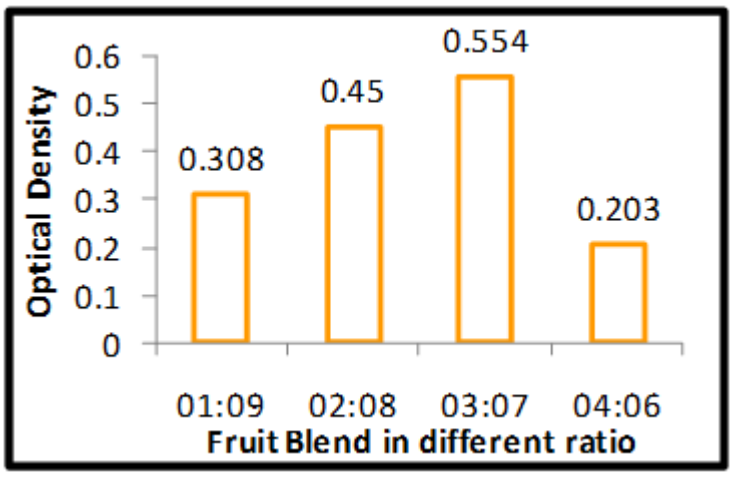

Fig-9: Difference in optical density of CAJ: Whey *B.lactis* before and after 24 h hours incubation

The OD variation before and after incubation was observed to be highest in 03:07 ratio for the fruit juice: non-dairy blend (0.547) and fruit juice:dairy blend(0.554) thus predicting the highest growth of *B. lactis* at this ratio.

Table-4: Change in turbidity of CAJ: Orange juice and CAJ:whey (*B.infantis*)

Blend ratios	Before incubation	After incubation	Difference in OD values before and after incubation
Cashew(10:00)	1.608±0.044	1.919±0.0005	0.268
CAJ: ORANGE BLEND RATIOS			
01:09	1.291±0.108	1.550±0.086	0.26
02:08	1.361±0.056	1.586±0.079	0.225
03:07	1.352±0.025	1.943±0.054	0.591
04:06	1.632±0.013	1.823±0.005	0.191
CAJ: WHEY BLEND RATIOS			
01:09	1.087±012	1.603±0.025	0.516
02:08	1.182±0.037	1.656±0.162	0.474
03:07	1.121±0.062	1.73±0.032	0.611
04:06	1.376±0.095	1.529±0.127	0.153

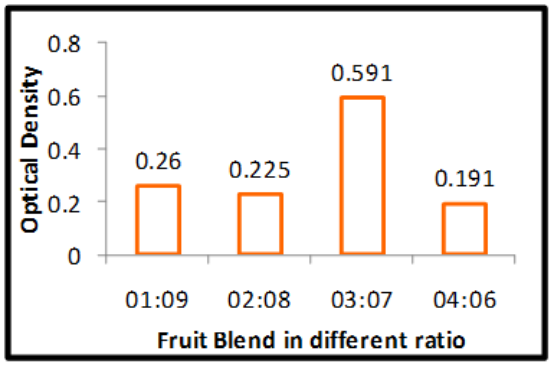

Fig-10: Difference in optical density of CAJ: Orange with *B.infantis* before and after 24 hours of incubation

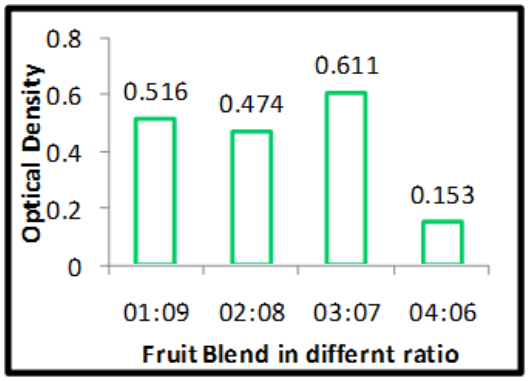

Fig-11: Difference in optical density of CAJ: Whey with *B.infantis* before and after 24 hours of incubation

The OD variation before and after incubation was observed to be highest in 03:07 ratio for the fruit juice: non-dairy blend (0.591) and fruit juice:dairy blend(0.611) thus predicting the highest growth of *B.infantis* at this blend ratio.

The observed results for both the probiotic cultures revealed that all blends could be utilized by Bifidobacterium sps as a substrate to promote growth. However results revealed that for both *B.lactis* and *B, infantis* the 30:70 blend ratio CAJ: Oranje juice and CAJ: whey showed maximum growth by plate count and turbidimetric method. A number of studies support the fact that fruits promote the growth of probiotics and several components in fruits can increase probiotic viability.

Fruit matrix contains several components that serves as a carrier for a probiotic and increases the probiotic cell viability. Fruits contain components such

as non digestible oligosaccharides like fructooligosaccharides [16] and other components such as xylitol, sorbitol, mannitol disaccharides[5], are rich in sugars, minerals, and vitamins, which can be used by probiotics [17] thus they act as potential probiotic source. Fruits, pulps and even the peels have been successfully incorporated into probiotic dairy products as sources of prebiotic fibers and nutrients that stimulate the growth and activity of intestinal microbiota. It has been reported that phenolic compounds and some organic acids present in the fruits are rapidly consumed by all the probiotic microorganisms and result in increased survival [18].

The cashew apple juice serves as a efficient medium for the growth of *L.casei* compared with a dairy medium. The viable cell count of L.casei increased to 8.00 log CFU/ml throughout the storage period. [19]. A probiotic beverage developed with orange and whey using L.acidophilus and B.bifidum had shown a maximum growth of total viable count of more than 106 cfu/ml after 24 hours of fermentation. [26] In this study, fermentation of pineapple juice with probiotic bacteria Lactobacillus and Bifidobacterium strains as well as changes of some properties in the beverage during storage were investigated. All tested strains exhibited good growth properties on pineapple juice without supplementation of any nutrient compounds. After 24 h fermentation, the cell counts of lactobacilli passed the level of $5*10^9$ cfu/ml, while the cell number of bifidobacteria reached a level of 10^9 cfu/ml [20].

From the above studies it is observed that fruits stimulate probiotic growth due to the presence of sugars, minerals, and vitamins,fibres,oligosaccharides and CAJ is a rich source of these[21] and hence has supported the growth of both *B.lactis* and *B.infantis* as observed in the results of this study.

Sensory Evaluation

Cashew apple juice is not consumed, mainly owing to its astringency and high perishability. But, the juice can be processed into various value added products to meet different end uses and can generate an additional income to cashew farmers [22]. Hence combinations of blends in dairy and non-dairy medium were done to improve acceptability and the results of the sensory evaluation are depicted below:

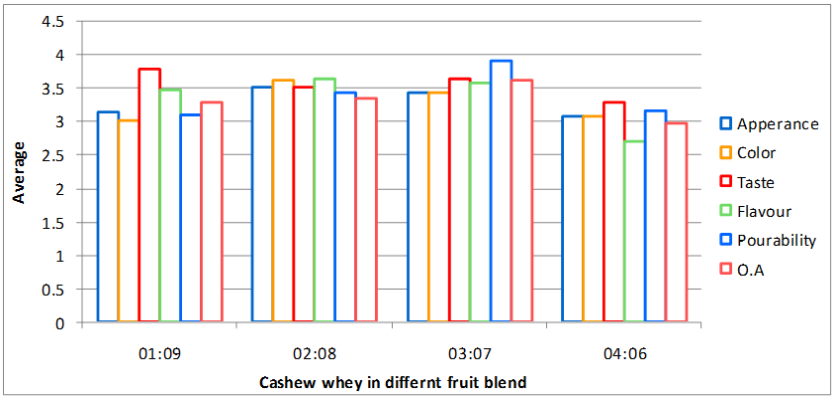

Fig-12: Sensory scores of cashew apple juice: whey in different blend ratio

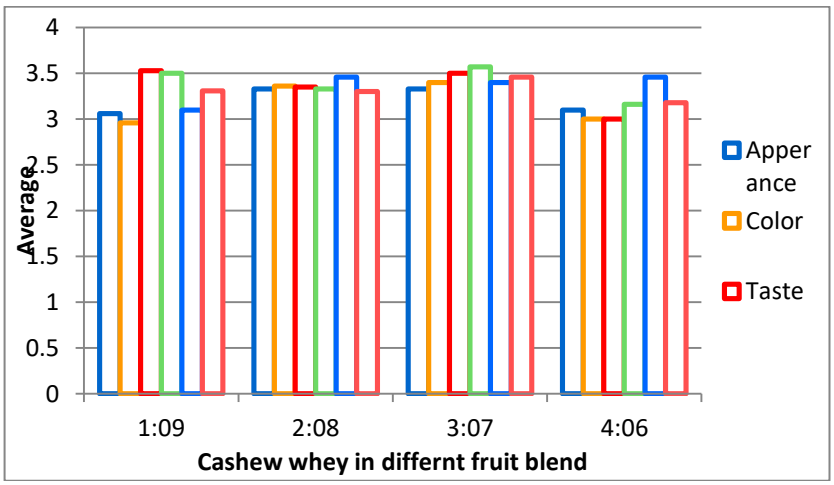

Fig-13: Sensory scores of cashew apple juice with orange juice in different blend ratios

Statistical analysis of the sensory results revealed that the overall acceptability scores of the blend ratios for both CAJ:orange juice and CAJ:whey differ significantly (P<0.05) and 30:70 ratio had the highest overall acceptability score 3.60 for CAJ: Orange juice and a score of 3.55 for CAJ: Whey and was rated best among others blends. Sensory attributes also revealed good overall acceptability of the cashew apple juice [23].

The sensory characteristics influence consumer's responses and it was observed in earlier studies that favourable results were obtained on evaluating sensory quality of sweetened probiotic cashew apple juice [24, 25].

Optimization of the ideal blend ratio to be used in the study

Based on the outcome of the probiotic growth, viability and sensory analysis the ideal ratio of cashew apple juice that can be blended into dairy/ non diary medium was observed to be 30 :70 and this blend ratio was optimized as the ideal synbiotic combination for further analysis. The 65:35 blend ratios of whey and orange juice fermented for 24 hr gave desirable results with highest sensory scores for overall acceptability and maximum viable counts in both fermented with *Lactobacillus acidophilus* and *Bifidobacterium bifidium*. There was no significant difference in results fermented with *Lactobacillus acidophilus* and *Bifidobacterium bifidium* but the results of probiotic beverage with *Bifidobacterium bifidium* showed a little better result as comparison to probiotic beverage fermented with *Lactobacillus acidophilus* [26].

Analysis of the ideal synbiotic combination (30:70 CAJ: Dairy/Non dairy medium) with 1.0% of probiotic culture during 4 hours of incubation.

Microbial growth measurement during incubation (*B.lactis and B.infantis)*

During 4 hours of incubation the growth rate of the sample were tested by plate count and turbidmetric method and the results are depicted below.

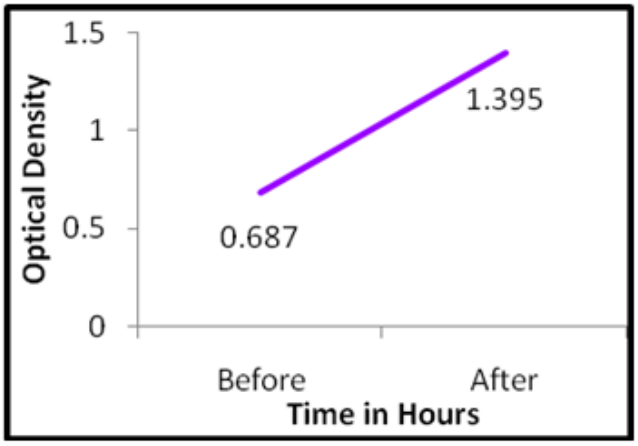

Fig-14: Change in optical density of CAJ: whey (*B.lactis*) in 4 hour

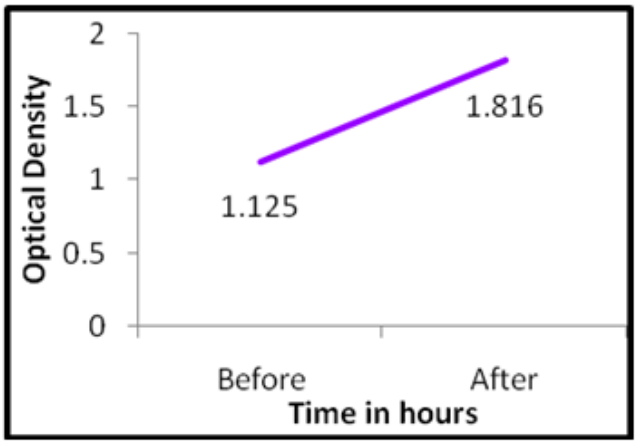

Fig-15: Change in optical density of CAJ: Orange (*B.lactis*) in 4 hour

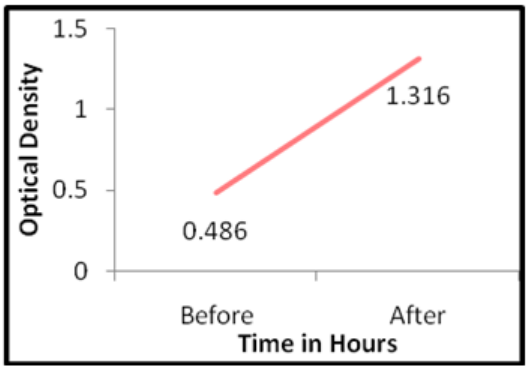

Fig-16: Change in optical density of CAJ: whey (*B.infantis*) in 4 hour

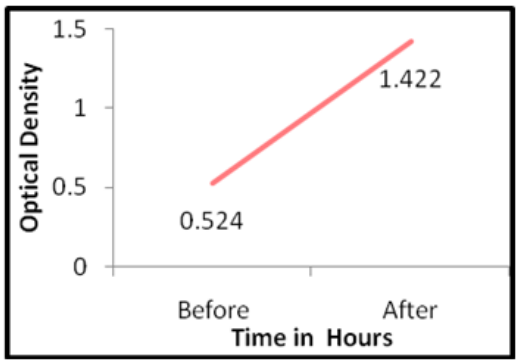

Fig-17: Change in optical density of CAJ: orange juice (*B.infantis*) in 4 hour

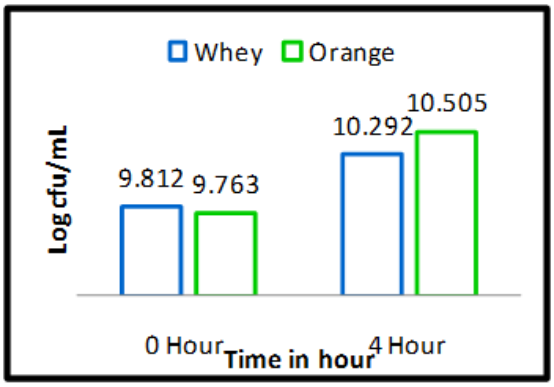

Fig-18: Prebiotic effect of CAJ in whey and orange (*B.lactis*) in 4 hour

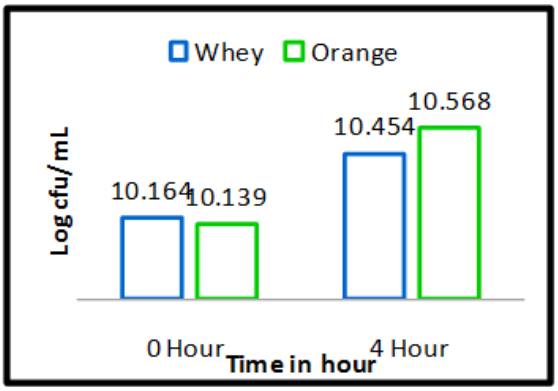

Fig-19: Prebiotic effect of CAJ in whey and orange (*B.infantis*) in 4 hour

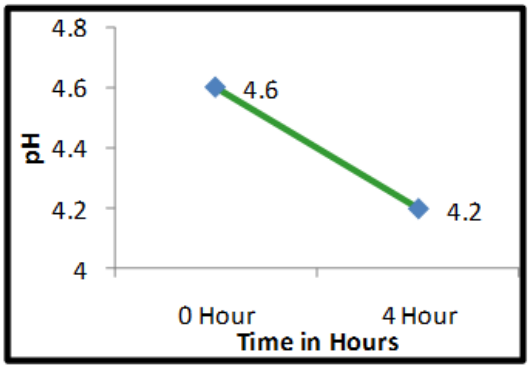

Fig-20: Change in pH of CAJ:Whey in 4 hours *(B.lactis)*

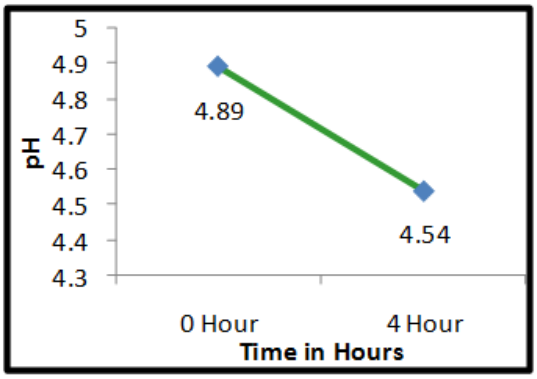

Fig-21: Change in pH of CAJ:Orange in 4 hours *(B.lactis)*

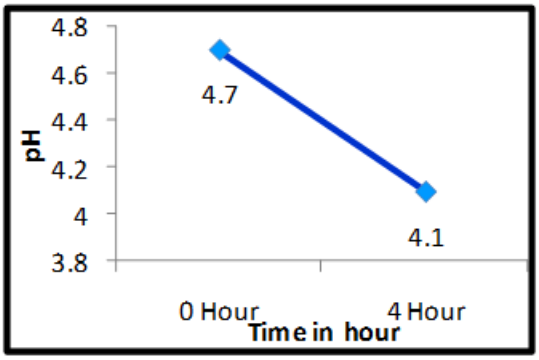

Fig-22: Change in pH of CAJ:Whey in 4 hours *(B.infantis)*

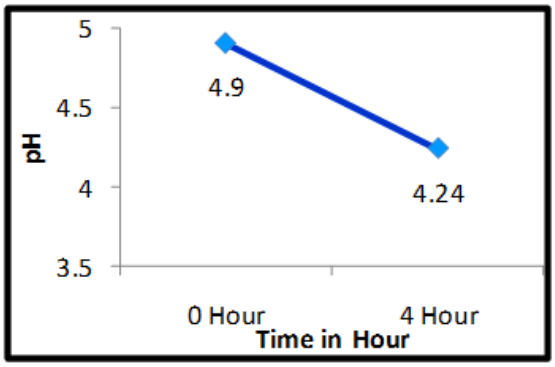

Fig-23: Change in pH of CAJ:Orange in 4 hours *(B.infantis)*

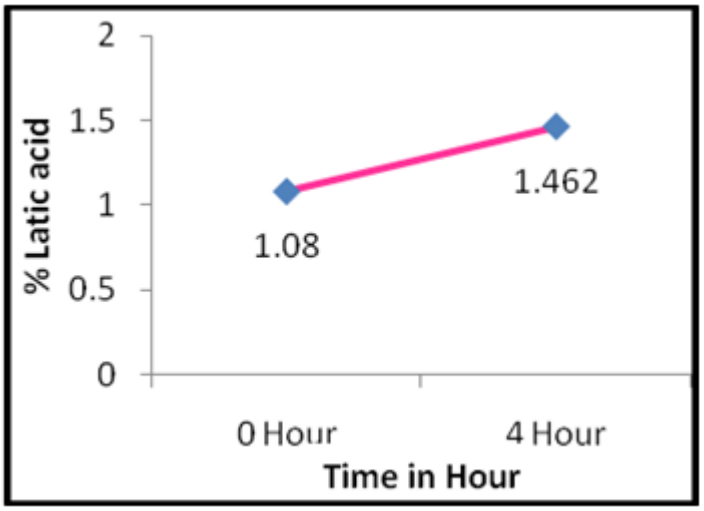

Fig-24: Change in Tiratable acidity of CAJ:Whey (*B.lactis*) in 4 hours

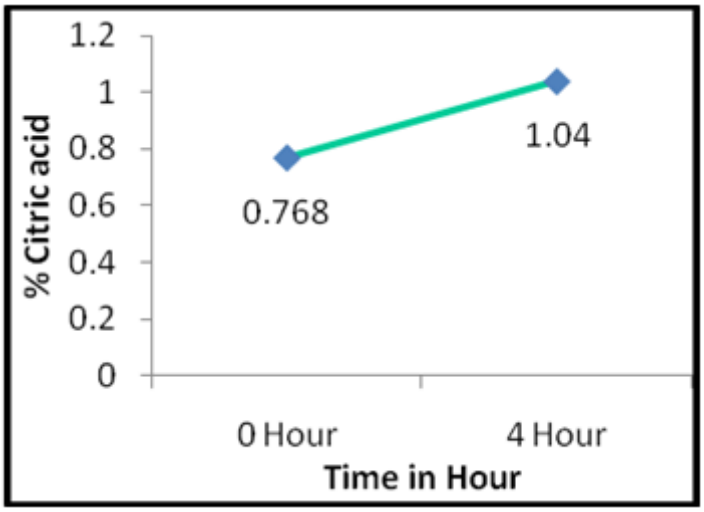

Fig-25: Change in Tiratable acidity of CAJ:Orange *(B.lactis)* in 4 hours

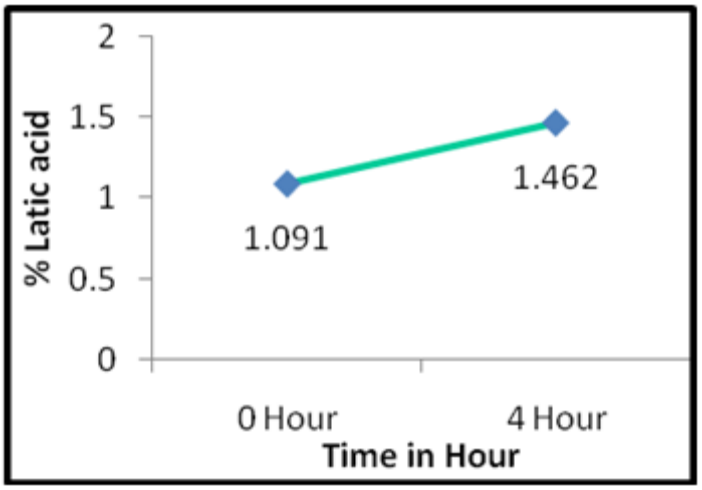

Fig-26: Change in Tiratable acidity of CAJ: Whey (*B.infantis*) in 4 hours

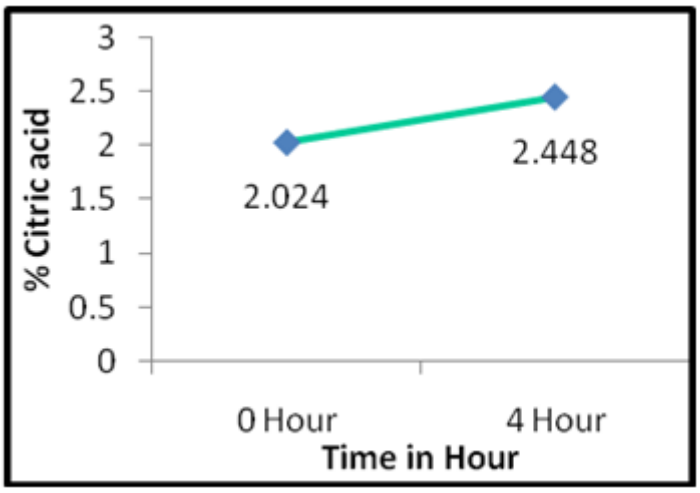

Fig-27: Change in Tiratable acidity of CAJ:Orange *(B.infantis)* in 4 hours

Researchers have observed in probioticated fruit juices, with the progress of fermentation time there is a significant increase in viable counts and titrable acidity increased significantly in the medium. The effect of incubation period on the pH of probioticated juice blends was analysed and it was observed that the pH of the samples reduced significantly with increasing fermentation time [27, 28, 9].The result observed in this study of increase in microbial growth, titrable acidity and decrease in pHwith the progress of fermentation is in concurrence with the previous studies.

Fruit juices and related beverages represent a suitable carrier for the delivery of probiotics [29]. A natural carbohydrate supplement and use of dietary bacteria is one approach to a healthy intestinal environment to confer a health benefit on the host. It is observed from the results in this study that CAJ in combination can be used for formulating synbiotic foods to promote gut well being and the ultilisation of CAJ will also decrease agricultural waste and provide economic gains.

SUMMARY

Consumer's beliefs and awareness in attaining wellness through food and the use of naturally functional foods to mediate and prevent diseases has paved the way for research in these areas. The results of the study done using cashew apple juice (CAJ) as prebiotic and Bifidobacterium sps as probitoics to obtain an ideal synbiotic combination was observed to be encouraging .From the results of the study it was observed that CAJ in both whey and orange juice at the 30: 70 ratio can be considered as a natural substrate to promote growth and viability of *B.lactis and B.infantis* and can be used as the ideal synbiotic combination for formulating foods that confer benefits on the host health.

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