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Probiotics Beverages of Sweet Lime and Sugarcane juices and its Physiochemical, Microbiological & Shelf-life Studies

Nazia Khatoon, Rajinder K Gupta

Abstract
The research was undertaken to estimate the suitability of Sugarcane and Sweet Lime juices as a sole source for growth of beneficial *Lactobacillus acidophilus.* Suitability of culture growth was also checked in various combinations which were prepared with herbs of Ashwagandha (*Withania somnifera*) and Green tea extract (*Camellia sinensis*), wheat grass juice, whey and oats. Juice samples were inoculated with 24-h-old lactic culture and incubated at 37 ºC. *Lactobacillus acidophilus* grew well in all these combinations and reached nearly upto 10^8 CFU/ml after 24 h of fermentation at 37 ºC. Sugarcane and Sweet Lime juice were also supplemented with 10 % & 5% v/v whey, with better growth of *Lactobacillus acidophilus*. After 3 weeks of storage at 4ºC, the viable cell counts of *Lactobacillus acidophilus* in the juices of Sugarcane control (SOC), Sugarcane juice with Wheat grass juice and *Withnia somnifera* (SOF1) and Sugarcane juice with green tea and whey (SOF2) were 4.0×10^8, 2.0×10^8 and 5.5×10^8 CFU/ ml, respectively. Culture viability in Sweet Lime juices was lost after 2nd week of storage because of the decrease in pH and higher acidity. Glucose (0.89%), sucrose (16.28%) and fructose (1.70%) was detected in Sugarcane juice and glucose (2.61%), sucrose (0.86%), fructose (2.70%) and maltose (0.38%) was detected in fermented Sweet Lime juice by HPLC analysis. Various secondary metabolites were detected in the SOF2 and CLF2 samples by GC/MS analysis. These juice samples were found phytochemically rich because of herb extracts. Fermented Sweet Lime and Sugarcane juice can be developed to perform as a functional probiotic beverage for vegetarians and lactose-allergic consumers.

Keywords: Lactobacillus acidophilus, probiotic, CFU/ ml, Withania somnifera

1. Introduction
Sensory acceptable and health, protecting probiotic juice has been considered by researchers in recent year. For large no of individuals are allergic from dairy products, thus there is an important requirement for non-dairy based probiotic products with an acceptable taste profile and proper shelf-life. Juices contain high amount of vitamins, mineral and polyphenolic compounds and are also free from allergens and easily available. Development of probiotic beverages along herbs without compromising on sensory image is an innovative idea for development of functional beverages. These beverages have been enriched with active components such as probiotics, prebiotics , phenolics, flavanoid and antioxidant Mousavi Z, *et al.* [1] The minimum number of live probiotic bacterial cells till the expiry date of the product should be around 10^7cfu/ml to attain maximum health benefits Nualkaekul & Charalampopoulos. [2] *Bifidobacterium* and *Lactobacillus* genus are most widely used probiotics Vitali, *et al*. [3] A number of health benefits had already proven by *Bifidobacterium* and *Lactobacillus* genus of micro-organisms like healing and prevention of antibiotic diarrhea, and also have the capacity to obstruct diabetes Roble *et al.* [4] Synthesis of B vitamins and folic acid, lowering intestinal pH Prado *et al.* [5] Anti-carcinogenic and anti-mutagenic activity Nosrati *et al*. [6] Lactose metabolism also improved and helps with the absorption of phosphor, copper, iron, manganese and calcium, zinc Rivera *et al*. [7] Dairy and cereals based probiotic product are more commonly available today Mollendorffet V *et al*. [8] An increasing number of vegetarian consumers all over the world demands for development of fruits and vegetable based probiotics products. Fermentation of juices from carrot Sharma and Mishra, [9] Tamminen *et al*., [10], beet root Czyżowska *et al*., [11] cabbage Yoon *et al*., [12] tomato Yoon *et al*., [13] cucumber Buruleanu *et al*., [14] and their mixtures, as well as banana Tsen *et al*., [15] orange Sheehan *et al*., [16] and other tropical fruits are reported Saw *et al*. [17] Lactic acid bacteria containing products like yogurt, curd, milk, etc are available in the large range of probiotic market. Fruit juices are not only, but they are also a good delivery system for

probiotics however, the low pH (2.5–3.7) and sensitivity of probiotic culture to acidic conditions, poses a challenge to its viability. It has been reported probiotic viability in fruit juices is affected by various conditions like strain, method of culture preparation, the physiological state of cells, storage temperature, oxygen level, and the presence of fibers Champagne, CP *et al*. [19]

2. Materials & Methods

2.1 Materials

Fresh Sugarcane juice, Sweet Lime juice and Organic wheat grass juice purchased from the local market and were stored under refrigeration condition. Green tea and Ashwagandha extract purchased from Shama Garg Plantea Pvt. Ltd, New Delhi, India and were stored in an airtight container. For the preparation of liquid whey, solution of citric acid (2%) was added to the boiling whole milk. The curdeled milk was filtered through muslin cloth to seperate the liquid whey and stored in an autoclaved bottle for further use.

2.1.1 Probiotic Strain

Lactobacillus acidophilus strain in the lyophilized form was obtained from Space Lab Pvt. Ltd, New Delhi.

2.1.2 Reagents

Folin Ciocalteu reagent, anhydrous sodium carbonate, aluminium chloride, potassium acetate, sodium hydroxide, butylated hydroxyltoluhene (BHT), gallic acid standard and quercetin standard were purchased from Sisco Research Labratories Pvt. Ltd, Mumbai, India. All the chemicals enlisted were of analytical grade. MRS broth, MRS agar, L-cysteine hydrochloric acid were purchased from Highmedia.

2.2 Starter Culture

Starter culture was prepared by incubating MRS-brith overnight at 37°C, supplemented with L-Cysteine (0.3%) for anaerobic conditions. The broth was further centrifuged (10,000 rpm, 10 min at 4 °C) and washed in saline water (0.2%) twice to obtain a culture.

2.3 Preparation of Sample

Non-dairy probiotic beverages with Sugarcane juice and Sweet Lime juice were prepared and Sugarcane juice and Sweet Lime juice were taken as controls.

2.4 *Citrus Limetta* Combinations

Freshly squeezed *Citrus limetta* juice heated at 80 ºC for the 20 min CLC (*Citrus limetta* Control) was used to prepare two different combinations. The juice sample was supplemented with 0.2% wheat grass juice and 0.1% Ashwagandha to prepare CLF1 (*Citrus limetta* Fortified 1). Second combination CLF2 (*Citrus limetta* Fortified 2) was prepared with 10 % liquid whey, 0.2% Green tea extract and 5% oats. All these combinations were used as a growth medium for *Lactobacillus acidophilus.*

2.5 *Saccharum officinarum* Combinations

Freshly Squeezed *Saccharum officinarum* juice heated at 80 ºC for 20 min was used as a Control *Saccharum officinarum* (SOC). *Saccharum officinarum* Fortified 1 (SOF1) was prepared with 2% Wheat grass juice and 0.1 % *Withania somnifera* extract. *Saccharum officinarum* Fortified 2 (SOF2) was prepared 10% liquid whey and 0.2% Green tea extract. All these combinations were used as a growth medium for the proper growth of probiotic strains.

2.6 Extract Preparation

For the extraction on shaker, 100ml of the sample with 100ml Acetic acid used as a solvent were taken in a reagent bottle and left on shaker (60 °C, 140 rpm) for 48 hours with successive filtration. Then, the crude viscous extract was procured by concentrating the filtrates using a hot air oven. After evaporation of organic solvents all extracts were stored at -20 ºC until analysis.

2.7 Bacterial viable count

Bacterial viable count (CFU/ml), was evaluated by the plate count technique. After a preparation of various dilutions with 0.2 % saline water, the samples were placed on MRS agar plates and incubated at 37 °C for 24h. The number of colonies obtained from various medium was used to calculate the number of viable bacteria in the medium.

CFU/ml= (No of colonies×Dilution factor) /Volume of Sample.

2.8 Preparation of growth curve

For an enumeration of bacterial population growth in the growth medium inoculation of viable cells into a sterile medium and incubation of the culture under optimum temperature and pH condition are required. Under these conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the, increase in cell numbers verses time of incubation. In the direct method number of colony forming unit with various time intervals were estimated and in the indirect determination method the optical density (OD) scales were estimated, that represents a doubling of turbidity.

2.9 HPLC Analysis

High Performance Liquid Chromatography (HPLC, Agilent Technologies, Inc., Santa Clara, United States) was used for the estimation of sugar concentration in the samples during the fermentation. A Supelcosil LC-NH2 column, 250×4.6 mm, 5 µm particle size (Supelco analytical, Sigma Aldrich Group, Taufkirchen, Germany) separates the sugars in 15 min using isocratic mobile phase acetonitrile/ water=75/25 %v/v at 40 °C (Muntean and Muntean 2010). A refractive index detector, also thermostated at 40 °C, was used for detection of the analytes, and the data were processed by Agilent ChemStation software. Muntean E and Muntean N. [20]

2.10 Lactic acid Content

The lactic acid content of the juice sample was determined by DGHS lab manual 5 test methods.

2.11 Antibacterial activity

All the Probioticated juice samples were screened for Antipathogenic activities by an agar well diffusion method

proposed by Kirby Bauer. The non probioticated juice was used as control in one of the wells. The antibacterial activities were screened by measuring the diameter (mm) of the zone of inhibition.

2.12 Effect of cold storage on cell viability in probiotic juice

After 24 hours of fermentation at 37 ºC, the samples of *Citrus limetta* and *Saccharum officinarum* and its various combinations (CLC, CLF1, CLF2, SOC, SOF1, SOF2) were stored at 4 ºC for 3 weeks. These samples were taken at weekly intervals, and the viability of probiotic cultures in probiotic juices was determined and expressed as colony forming units (CFU/ml).

2.13 physicochemical analysis

All the physical and physicochemical analysis was performed at every 24 hours of culture growth stage. Physicochemical parameters play significant role in the survival of probiotic culture so it is important to estimate them. Various parameters like pH, total soluble solids and acidity were determined by pH meter, digital Refractometer and titration respectively.

2.14 Phytochemical analysis

2.14.1 Total Phenolic Content

The whole phenolic content was estimated according to the Folin-Ciocalteau method given by McDonald S *et al.* [21]

2.14.2 Total flavonoid content

Flavonoid content of the samples determined by Aluminium chloride method Chang, C *et al.* [22] Catechin (1 mg/ml) was used as a standard for the calibration curve and the flavonoid content was expressed as µg of catechin equivalents (CE) per mg of the extract.

2.15 Antioxidant Potential

The antioxidant potential can be assessed by finding their free radicals scavenging capacity or their potential to reduce the compounds using the DPPH assay.

2.15.1 DPPH Method

Radical scavenging activity was estimated by the method given by Blois 2000 with slight modification. Initially DPPH solution was prepared of 0.3 mM concentration by dissolving 1.1829 mg in 10 ml methanol. Then 3 ml of mixture was prepared by 1ml DPPH, 1 ml extract (different conc.) and 1 ml methanol, the solutions were kept in dark for 10 min. The absorbance was taken at 517 nm. Methanol was used as a reference sample. The % inhibition was determined by:

% Inhibition= ([B-A]) /B × 100
Where B= OD of Blank; A= OD of Sample
Ascorbic used as a standard.

2.16 Nutritional analysis

A final product combination which was best in its color, flavor and sensory test were subjected to nutritional analysis. The Moisture content determined by method given by Khatoon N *et al.* [23] using a hot air oven (105 ºC for 6 hours). Ash content estimated by using Muffle furnace (550 ºC for 6 hours) and calculated by weight difference. Protein content was obtained using Kjeldal method Ref. 976.05 Arlington, VA. [24] Crude fat content of raw sample screened by using petroleum ether as an extracting medium. Dietary fiber content was estimated by IFS/C/SOP/FC/007/(AOAC 993.21) method.

Total carbohydrate of fermented juice samples was calculated using this formula

Carbohydrate content = {100 - moisture (%) - protein content (% dry weight) - crude fat (% dry weight) - ash (% dry weight)}

The calorific value per 100 ml was calculated according to the system of Atwater (in kcal) using the formula:

Energy (kcal) = (3.36 X % protein) + (3.60 X % carbohydrate) + (8.37 X % fat)

2.17 Characterization of secondary metabolites using GC/MS:

Mass spectrometric detector (MSD- an Agilent 5975B) was used in scan mode (m/z 35-1050). The automatic RTL screener software in combination with the Agilent NIST'05 library was used to screen, volatile and semi volatiles Mehra M *et al*. [25]

2.18 Sensory evaluation

2.18.1 Hedonic method for smell and taste:

Each panelist was asked to taste each sample in turn and tick a box, from 1 dislike very much to like very much to indicate their preference. A 9-point-scale is used. The tester may also wish to make remarks about the product appearance, taste, odor and texture. The results were analyzed according to their score Gatti *et al.* [26]

3. Results and Discussion

Lactobacillus acidophilus was found to be capable of growing well in sterilized juice of Sweet Lime and Sugarcane. The Lactic acid fermentation was performed in various combinations of these juices which prepared by herbal extract, oats and whey.

3.1 Viability (CFU/ml) of *Lactobacillus acidophilus* in various juice combinations:

L. acidophilus, was capable of utilizing Sugarcane juice and Sweet Lime juices for growth and lactic acid production. The viable cell counts of *L. acidophilus* cultures reached 10^8 CFU/ml after 24 h of fermentation at 37ºC. The Results indicated that the juices served as a good medium for growing Probiotics. According to the Codex Alimentarius standard, a commercial probiotic beverage should possess a minimum viable count of 10^6 CFU/ml at the time of consumption David, H *et al.* [27] *L. acidophilus* was growing well and capable of utilizing pasteurized *Citrus limetta* and *Saccharum officinarum* for their growth without any nutritional supplementation. *L. acidophilus* was also investigated into other combinations of Sweet Lime and Sugarcane juice CLC, CLF1, CLF2 and SOC, SOF1, SOF2. Both these combinations were also shown as a significant growth medium for the proper survival of *L. acidophilus.* Sugarcane juice was found to be a better

fermented product than the other substrates which might probably due to higher moisture and sugar content.

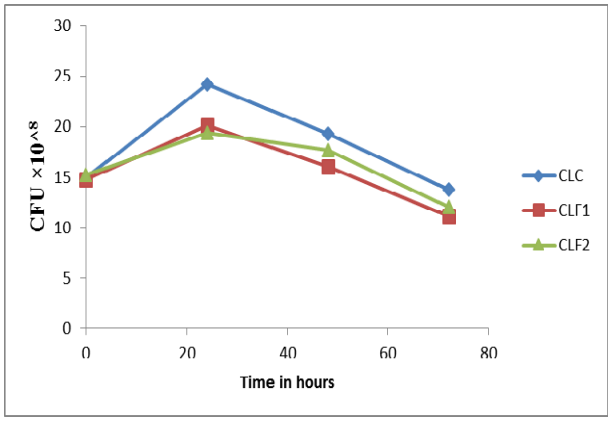

Fig 1: Growth pattern of *Lactobacillus acidophilus* in Sweet Lime juices

Table 1: Viability (CFU/ml) of *Lactobacillus acidophilus* in Sweet Lime juice

Time In Hours	Viability (CFU/ml) of Lactobacillus acidophilus		
	(CLC)	(CLF1)	(CLF2)
0	14.9×10^8	14.8×10^8	15.2×10^8
24	24.2×10^8	20.1×10^8	19.4×10^8
48	19.3×10^8	16.0×10^8	17.7×10^8
72	23.8×10^8	11.1×10^8	12.0×10^8

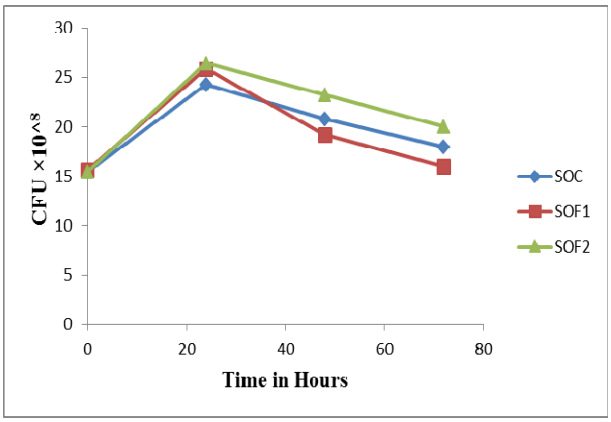

Fig 2: Growth pattern of *Lactobacillus acidophilus* in Sugarcane juices

Table 2: Viability (CFU/ml) of *Lactobacillus acidophilus* in Sugarcane juice

Time In Hours	Viability (CFU/ml) of Lactobacillus acidophilus		
	(SOC)	(SOF1)	(SOF2)
0	15.4×10^8	15.6×10^8	15.3×10^8
24	24.3×10^8	24.9×10^8	26.4×10^8
48	20.8×10^8	19.2×10^8	23.2×10^8
72	18.0×10^8	16.0×10^8	20.0×10^8

3.2 Growth pattern of culture in MRS broth

A growth pattern observed in MRS broth was depicted and the exponential growth pattern was observed. The broth was supplemented with 0.3 % of L-cysteine to create anaerobic conditions for proper growth of probiotics.

3.3 Sugar Content by HPLC

Content of sugar, estimated in the Probioticated fortified Control juice of *Citrus limetta* and *Saccharum officinarum* juice. After 24 hours of fermentation no very significant change in sugar content was observed in the juice sugar content**.** Mainly present sugar in *Saccharum officinarum* juice is glucose, sucrose and fructose and their content after fermentation are 0.89, 16.28 and 1.70 (g/100g) respectively maltose didn't detect in Sugarcane juice. The main sugar content of Sweet Lime juice is glucose, sucrose, fructose and maltose their content after fermentation are 2.61, 0.86, 2.70 and 0.38 (g/100g) respectively. By observing initial concentration of sugar content it was observed that culture consuming them in small quantities.

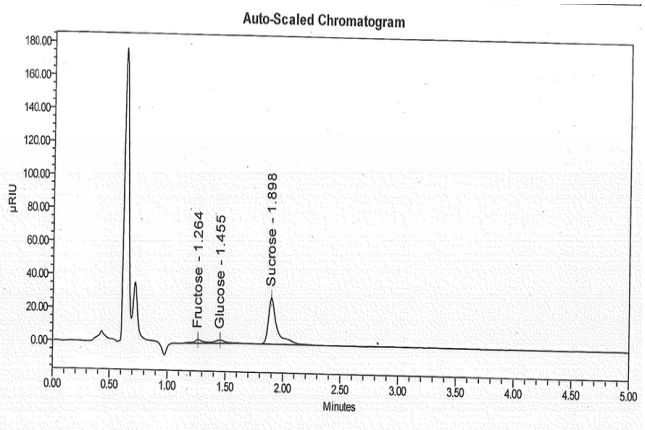

Fig 3: Chromatogram of HPLC for Sugarcane juice (SOC).

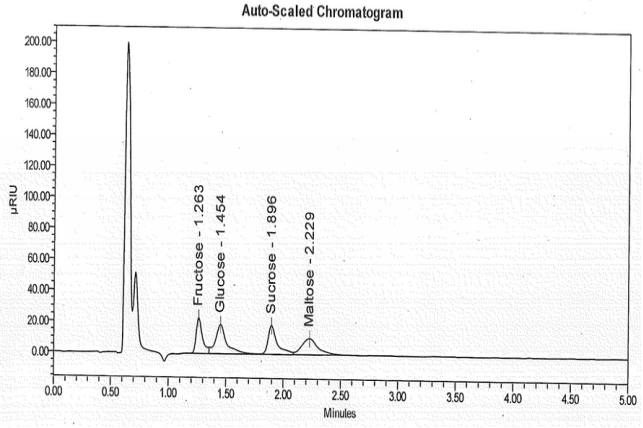

Fig 4: Chromatogram of HPLC for Sweet Lime juice (SOC).

3.4 Antimicrobial activity

Two gram positive (*Bacillus subtilis and Bacillus cereus*) and three gram negative (*Escherichia. coli, Shigella flexneri and Salmonella enterica*) bacterial test pathogens were used to evaluate the antibacterial activity of the fermented Juices of *Saccharum officinarum* and *Citrus limetta*. Agar well diffusion method was used to access the activity against the bacteria test pathogens. Results were analyzed by measuring the zones of inhibition. Significant zones of inhibition (in mm) were observed against all test pathogens. In conclusion the probioticated juices could serve in their antagonistic activities against the pathogens which could be due to the metabolite

secreted by the lactic acid bacteria specially type of organic acids.

Table 3: Antimicrobial Activity of Probioticated juice against pathogens

Test Pathogens	Diameter of zone of inhibition (mm)	
Gram Positive	CLC	SOC
B. subtilis	3	6
B. cereus	8	5
Gram Negative		
E.cole	14	8
S. flexneri	16	14
S. enterica	10	5

3.5 Effect of cold storage on cell viability in probioticated juices and its combinations:

The results illustrate the effect of cold storage on the viability of lactic acid bacteria in juice samples and indicated that LAB was capable of surviving in the fermented Sugarcane juice at 4 ºC for several weeks but not able to survive in the Sweet Lime juice. The viable cell counts of *L. acidophilus* were 4.0×10^8, 2.0×10^8 and 5.0×10^8 respectively in Sugarcane juice, after 3 weeks of storage at 4 ºC. However, *L. acidophilus* was unable to survive in Sweet Lime juice after first week of storage because of significant drop in the initial pH. It was reported that acid production ability of lactic acid bacteria, especially during post-incubation (postacidification), affects the cell viability of probiotic bacteria. All the assessment on the effect of the cold storage of sample was performed under very sterile and controlled conditions to prevent contamination of juices.

Table 4: Effect of cold storage on the viability of lactic culture in fermented Sweet Lime juice

Time In Week	Viability (CFU/ml) of Lactobacillus acidophilus		
	Citrus limetta (CLC)	Citrus limetta (CLF1)	Citrus limetta (CLF2)
0	23.8×10^8	11.1×10^8	12.0×10^8
1	18×10^8	9.60×10^8	10.9×10^6
2	ND	ND	7.6×10^5
3	ND	ND	ND

Table 5: Effect of cold storage on the viability of lactic culture in fermented Sugarcane juice

Time In Week	Viability (CFU/ml) of Lactobacillus acidophilus		
	Saccharum officinarum (SOC)	Saccharum officinarum (SOF1)	Saccharum officinarum (SOF2)
0	18.0×10^8	16.0×10^8	20.0×10^8
1	12.0×10^8	11×10^8	17.3×10^8
2	9.0×10^8	8×10^8	11×10^8
3	4.0×10^8	2.0×10^8	5.5×10^8

3.6 Various parameters

3.6.1 Change in pH of Probioticated juice

Fermentation time 24hr results in a significant increase in viable counts (CFU/ml) and reduced the pH of sugarcane juice from an initial value of 5.03 to lower than 4.99 and Sweet Lime juice pH was reduced from an initial value of 3.59 to lower than 3.36. pH and growth of probiotic culture are much related to each other. The graph showed the change in pH of *Saccharum officinarum* juice with time**.** The most intensive growth was observed at pH of 5.27 in *S. officinarum* juice.

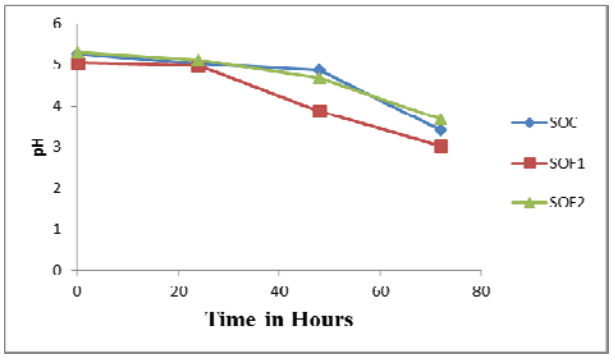

Fig 5: Change in pH Value of Sugarcane juice This graph below shows the change in the pH value of *Citrus limetta* juice during the subsequent period of time.

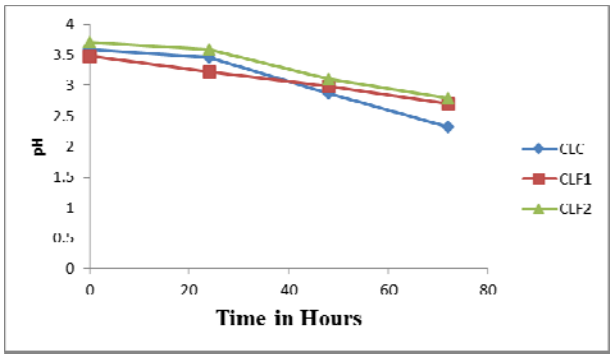

Fig 6: Change in pH Value of Sweet Lime juice

3.6.2 Total soluble solid

The TSS or sugar content, measures the carbohydrates, organic acids, proteins, fats and minerals of the fruit. TSS is determined by the index of refraction. Brix is widely used to determine the concentration of sugar in a product. Degree Brix usually consider equivalent to sugar in solution. Slight decrease in TSS value was observed after 7 days of storage.

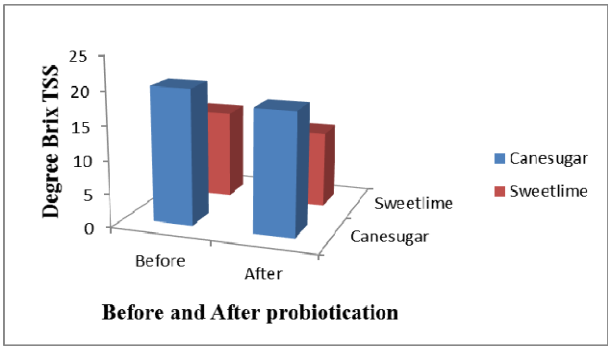

Fig 7: Change in TSS Value

3.6.3 Lactic acid content

Lactic acid produced after the 24 hours of the fermentation was examined for the Sugar Cane and Sweet Lime juices without any fortification. CLC and SOC showing 0.77% and 0.91% respectively.

3.6.4 Acidity Value

Citric acid content was measured after 24 hours of fermentation of juices. Acidity influences the palatability of various juices. Result (%) shows that Sweet Lime juice (CLC) and its combinations CLF1 and CLF2 have 0.82, 0.89 and 0.78 % citric acid respectively. Sugarcane juice (SOC) and its combinations SOF1 and SOF2 have 0.38, 0.24 and 0.30 % citric acid.

3.7 Phytochemical analysis

3.7.1 Extraction and quantification of phenols in probioticated juice sample

Phytochemicals, mainly phenolics are considered to be the important bioactive compounds for health benefits. The extracts which contain different classes of polyphenols are not only attractive in Phytotherapy but also in the food industry.

3.7.2 Total phenolic content

Total phenolic content of the extract of probioticated juice samples was found by the equation y =0. 0151x+0.191, R^2 =0. 9986 based on the calibration curve of gallic acid standard. The amount of phenolics was found in terms of µg of gallic acid equivalents per 100 mg of the extract. CLF2 and SOF2 extract contains 5.78 and 6.73 GAE (µg) /1 mg respectively.

3.7.3 Total flavanoid content

The results were expressed in µg of catechin equivalent (CE) per 1 mg of the extracts. Flvonoid content was calculated using the equation y = 0.0002x+0.0475, R^2=0. 9773 based on the calibration curve of catechin as the standard. The results shows, CLF2 and SOF2 extract contain 8.74 and 11.08 CE (µg/mg) respectively.

3.7.4 Antioxidant potential

Polypenolics are considered to function as antioxidants by various mechanisms like radical scavenging by H-donation, prevention of chain inhibition by donating electrons. Hence DPPH assay was performed to find out the potential of the probioticated juice sample as a candidate for nutraceutical and determine its parmacoloical significance.

3.7.5 DPPH Activity

The DPPH free radical scavenging capacity of the unfermented fresh Sweet Lime juices was 18%. In the case of fresh Sugarcane fermented with *L. acidophilus* DPPH radical scavenging activity was 21%, while after fermentation reduction in scavenging capacity were observed. After of fermentation with *L. acidophilus* DPPH radical scavenging activity of fermented Sweet Lime and fermented Sugarcane was 12 % and 16 %, respectively was observed on the 7th day of storage.

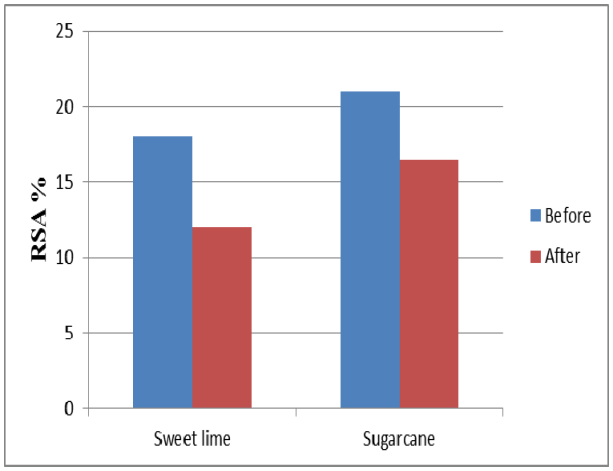

Fig 8: RSA (%) of control juice sample of Sweet Lime and Sugarcane

3.8 Nutritional Analysis

Juice of Sugarcane is well known as a wonder juice because of its appropriate nutritional composition**.** Sugarcane juice is a vital source of vitamins and minerals. Various vitamins like Riboflavin, Pantothenic acid and Niacin are present in considerable amount which play important role in body metabolism. Some important properties that make it a wonder juice is its low Glycemic index, high alkaline content, contain energy giving growth inducing nutrient, low in calorie and excellent antioxidant properties. In this report estimation of juice nutritional profile after probiotication were reported as follows. *Citrus limetta* is a species of citrus. Their description is similar to lemons. It contains higher amount of ascorbic acid Sandhu KS & Minhas KS. [28]

Table: 6 Nutritional analysis of juice of Probioticated Fortified *Citrus limetta* juice (FCL2) (g/100g**)**

Components	Proximal Value (%)	
	CLF2	SOF2
Moisture	68.92	71.19
Protein	2.74	0.94
Dietary fiber	ND	0.87
Carbohydrate	24.25	28.70
Fat	3.20	0.12
Energy (Kcal)	123.28	107.16

3.9 Characterization of secondary Metabolites of the extracts using GC/MS

Phytochemical screening of different extracts was done using GC/MS to determine the composition of fruit sample. The data obtained by marching against the NIST, 05 a library revealed the presence phytotherapeutically important metabolites in extract of the probioticated juice extract are shown in the table below.

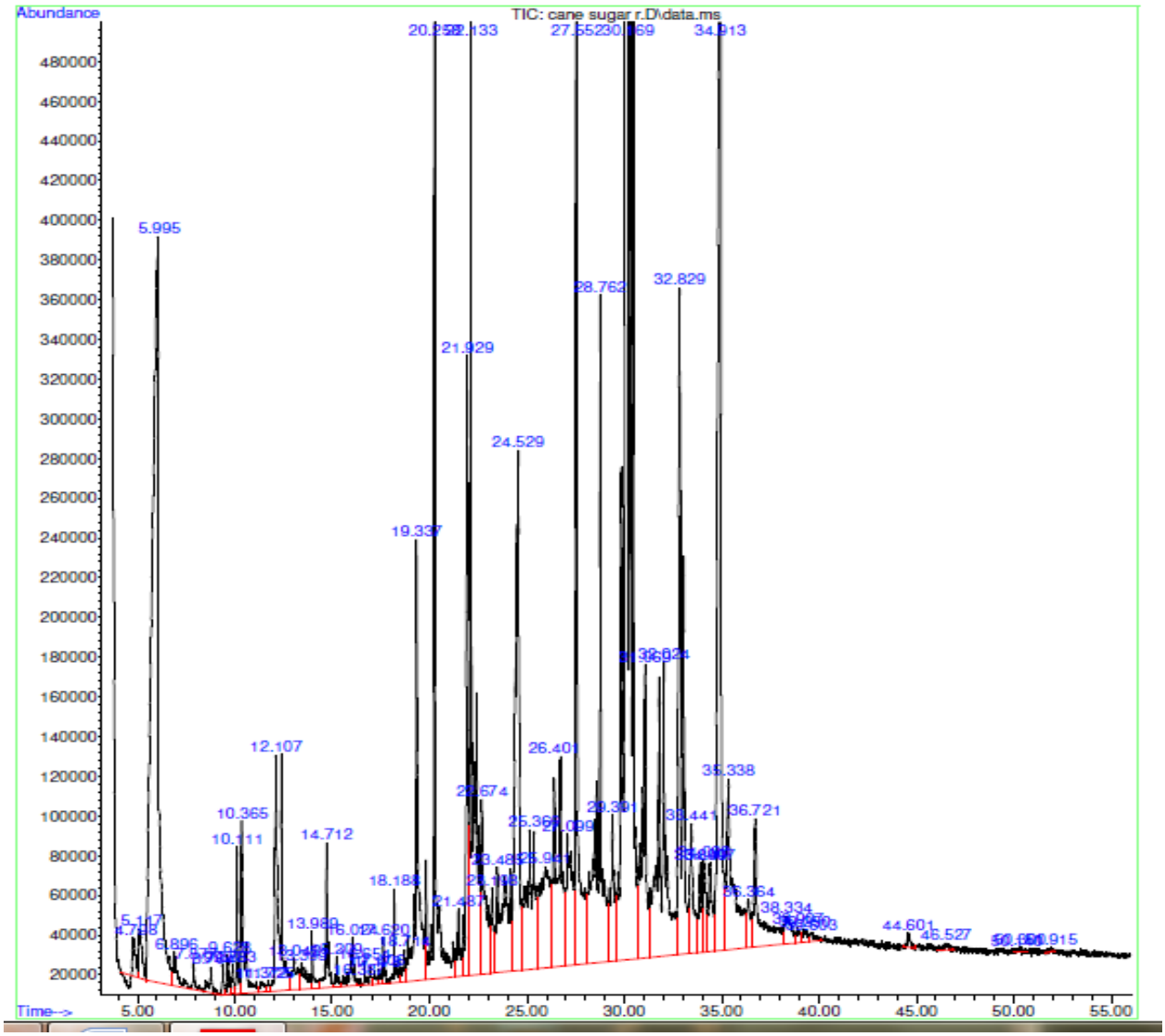

Fig 9: Chromatogram of GC-MS for Fortified Sugarcane juice Sample (SOF2).

Table 7: List of compounds detected in the probioticated juice of *S. officinarum* (SOF2)

S.No.	Compound Detected	CAS#	Area (%)	R.T
1.	Tetradecanoic acid	000544-63-8	0.21	18.183
2.	1H-Purine-2,6-dione,3,7-dihydro-1,3,7-trimethyl-	000058-08-2	1.89	19.338
3.	n-Hexadecanoic acid	000057-10-3	3.08	20.258
4.	9,17-Octadecadienal, (Z)-	056554-35-9	2.08	21.929
5.	9-Octadecenoic acid, (E)-	000112-79-8	2.08	21.929
6.	Octadecanoic acid	000057-11-4	3.35	22.131
7.	Stigmasterol, 22,23-dihydro-	1000214-20-7	3.35	24.531
8.	γ-Sitosterol	000083-47-6	3.35	24.531
9.	β-Sitosterol	000083-46-5	3.35	24.531
10.	Heptacosane, 1-chloro-	062016-79-9	1.70	25.361
11.	Nonacosane	000630-03-5	8.02	27.548
12.	Tetracosane	000646-31-1	8.02	27.548
13.	Eicosane	000112-95-8	3.32	28.759
14.	Hexadecane, 1-iodo-	000544-77-4	3.32	28.759
15.	Vitamin E	000059-02-9	1.90	31.069
16	Campesterol	000474-62-4	3.44	32.830
17.	5-Cholestene-3-ol, 24-methyl-	1000214-17-4	3.44	32.8303
18.	Stigmasterol, 22,23-dihydro-	1000214-20-7	7.32	34.916
19.	γ-Sitosterol	000083-47-6	7.32	34.916
20.	β-Sitosterol	000083-46-5	7.32	34.916

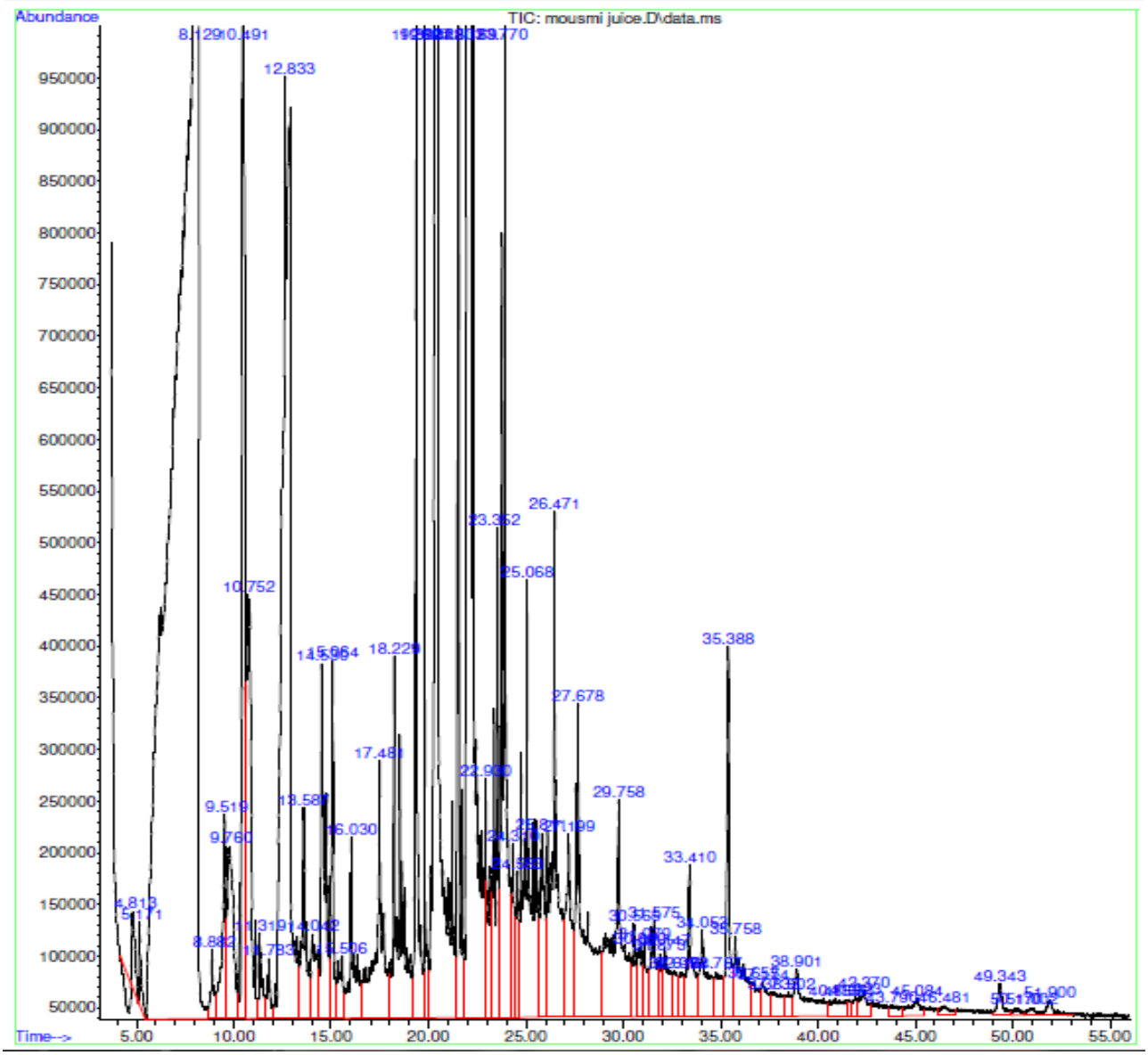

Fig 10: Chromatogram of GC-MS for Sweet Lime juice Sample (CLF2)

Table 8: List of compounds detected in the probioticated juice of *C. limetta* (CLF2)

S.No.	Compound name	CAS#	Area (%)	RT
1.	Tetradecanoic acid	000544-63-8	1.43	18.228
2.	1H-Purine-2,6-dione, 3,7-dihydro-2,3,7-trimethyl-	000058-08-2	2.13	19.392.134
3.	Hexadecanoic acid, methyl ester	000112-39-0	0.77	19.831
4.	Pentadecanoic acid, 14-methyl-, methyl ester	005129-60-2	0.77	19.831
5.	n-Hexadecanoic acid	000057-10-3	9.29	20.448
6.	9-Octadecenoic acid (Z) -, methyl ester	000112-62-9	1.77	21.503
7.	9,12-Octadecadienoic acid (Z, Z)-	000060-33-3	17.09	22.164
8.	6-Tetradecyne	003730-08-3	17.09	22.164
9.	Cyclohexane, 1-(1,5-dimethylhexyl) -4-(4-methylpentyl)-	056009-20-2	0.72	22.927
10.	Cycloeicosane	000296-56-0	0.72	22.927
11.	3-Eicosene, (E)-	074685-33-9	0.72	22.927
12.	7-Pentadecyne	022089-89-0	0.38	24.587
13.	9-Octadecenal, (Z)-	002423-10-1	2.12	26.471
14.	Vitamin E	010191-41-0	0.46	31.574
15.	Campesterol	000474-62-4	0.59	33.413
16.	5-Cholestene-3-ol, 24-methyl	1000214-17-4	0.59	33.413
17.	Stigmasterol, 22,23-dihydro-	1000214-20-7	0.92	35.387
18.	β-Sitosterol	000083-46-5	0.92	35.387

A number of secondary metabolites found in the sample extract like β-Sitosterol, Campesterol and γ-Sitosterol, these are plant Sterols. The cholesterol lowering effects of dietary plant sterols are proven by research Racette BS, *et al.* [29] Besides plant sterol Vitamin E, Hexanoic acid and other plant polyphenols were also detected in the samples.

3.10 Preliminary sensory evaluations

The preliminary sensory evaluation was performed to examine the acceptance of probioticated juice by consumer against its color, odour and overall acceptability parameters. According to the panelists, the changes in the smell by fermentation was significant. Color of the fermented Sugarcane juice was slightly darkened, but still attractive to the eye. The juice with 5% v/v whey had a slight milky taste and its transparency was lesser than the other combination.

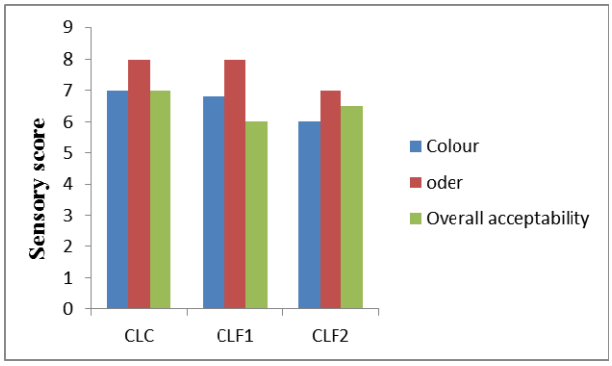

Fig 11: Sensory score of color, odor and overall acceptability of *C. limetta* Control (CLC), *C. limetta* Fortified 1 (CLF1), and *C. limetta* Fortified 2 (CLF2) after 7 days of storage.

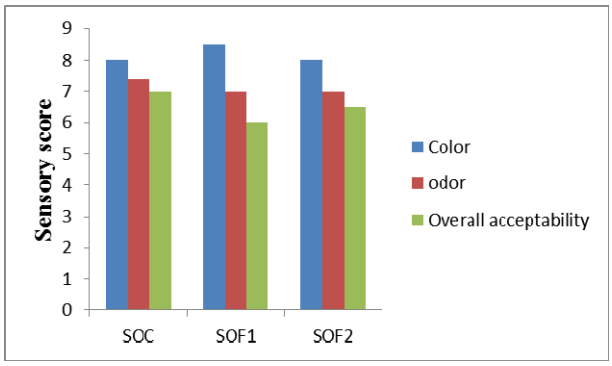

Fig 12: Sensory score of color, odor and overall acceptability of *S. officinarum* Control (SOC), *S. officinarum* Fortified 1 (SOF1), and *S. officinarum* Fortified 2 (SOF2) after 7 days of storage

4. Conclusion

The research indicated that the proven probiotic strain of *Lactobacillus acidophilus* was able to survive and capable of rapidly utilizing the nutrients of all control and fortified juice samples for cell synthesis and lactic acid production. Herb extract increases its functional properties. Sweet Lime juice and Sugarcane juices were used as a sole source of nutrition for probiotic fermentation, which shows prolific growth of *L. acidophilus* culture. Furthermore, physiochemical parameters like pH, TSS and acidity were found suitable for growth of culture in both juice samples. Shelf-Life study in Sugarcane juice revealed that *L. acidophilus* culture was survived throughout the refrigeration period of 3 weeks while fast reduction in viability was observed in the Sweet Lime juice during storage. Excess decline in pH and high acidity were observed in the Sweet Lime juice. Antioxidant activity was also found high in both samples. γ- Sitosterol, β-Sitosterol, Campesterol, Stigmasterol and various plant sterol presence were detected in GC/MS analysis whom responsible for cholesterol lowering effect. Sugar profile study by HPLC have shown a slight decrease in sugar content after probiotication which means culture utilizing sugar for its growth. 0.77 and 0.91% of lactic acid production were observed fermented juice samples of Sugarcane and Sweet Lime. The juice CLF2 & SOF2 which were supplemented with 5% & 10% liquid whey yielded the best fermentation results in terms of highest viability as well as refrigeration storage stability. High amount of antipathogenic activity was observed in all probioticated samples which proves its ability to decline intestinal pathogens. This kind of research can facilitate the development of new fermented, non dairy, nutritionally well balanced and sensory acceptable probiotic products for vegetarians and consumers who have allergies to dairy products. Furthermore, juices based probiotic products would offer bioactive characteristics which are lacking in a typical dairy based probiotic products.

5. Acknowledgement

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