present investigation it was important to analyse the chemical properties of the sweet orange fruit to study the nutritional and chemical changes in the juice due to microbial activity and storage. The Total Soluble Solids (TSS) of probiotic juice, was determined using a hand refractometer (ERMA) corrected at 20 °C. The pH of the juice was measured using an electronic digital pH meter. Percent acidity was determined by titrating the sample against 0.1 N NaOH using phenolphthalein as indicator. Estimation of total sugars was performed by phenol sulphuric acid method and reducing sugars was determined by Nelson-Somogyi method. The non-reducing sugar was obtained by subtracting reducing sugars from total sugars. Ascorbic acid contents of the samples were obtained using 2, 6-dichlorophenol indophenol method.

Encapsulation of Probiotic Strains

Encapsulation of strains was done by extrusion method (Kore and Chakraborty, 2005). Probiotic culture at 10% (v/v) of the final juice was encapsulated using a combination of sodium alginate and guar gum at 1 and 0.8% (w/v) respectively. The cell suspension contained about $4.3x10^9$ cells per ml prior to encapsulation.10 ml of the probiotic culture was mixed with sterile sodium alginate-guar gum solution at a ratio of 1:2 and the mixture was placed in a sterile syringe and dropped into 0.3 M calcium chloride solution. Interaction between the two solutions led to formation of beads and the resultant beads were the resulting beads were then stored in 0.1% peptone solution at 4 °C.

Inoculation into Substrate

For preparation of probiotic sweet orange juice with free strains, the probiotic culture is added to the juice at 10% (v/v) inoculum level and incubated at 37 °C for 10 hrs. For preparation of probiotic juice with encapsulated strains, the washed beads were aseptically added to the pasteurized fruit juice and incubated at 37 °C for 10 hrs. Post incubation the probiotic juice samples were stored at 4 °C for a period of 4 weeks.

Organoleptic Evaluation

The sensory acceptance of probiotic sweet orange juice with free strains and encapsulated strains was performed after incubation and also at weekly intervals during storage at 4 °C.The sweet orange juice samples were evaluated for their sensory characteristics namely, color, taste, flavor and overall acceptability by a trained panel. A panel of 20 members were asked to rate the product on 9 point Hedonic scale with corresponding descriptive terms ranging from 1 = dislike very much, to 5 = neither like nor dislike, to 9 = like extremely well, i.e., higher sensory score indicated better overall acceptance. 9 'like extremely' to 1 'dislike extremely'.

Cell Viability Count

The viability of probiotic cells during storage is of paramount importance because for a probiotic food to confer health benefit the number of cells should be > 10^7 cfu/ml or gm at the time of consumption.Viable cell count of juice with free cells was analyzed at weekly intervals by the Standard Plate Count (SPC) method with MRS medium at 37 °C for 48 hrs. To determine the viability of encapsulated strains in the juice, the enumeration was done by releasing the entrapped strains from the capsules using the method suggested by Sheu and Marshal (Sandhu and Singh, 2001). The capsules were depolymerized by using a solution (28 ml of 0.2 M NaH_2PO_4 and 72 ml of 0.2 M Na_2HPO_4 adjusted to 200 ml with distilled water, pH 7.1 ± 0.1, sterilized). After incubation at 37 °C for 10 min, the mixture was vortexed at high speed for breaking the polymer and releasing completely the encapsulated culture into the buffer. The released cells were enumerated using MRS media at 37 °C for 24-48 hrs.

RESULTS AND DISCUSSION

Chemical Analysis

The samples were analysed quantitatively for their chemical compositions before and after probiotication. The data of various parameters viz. total soluble solids, pH, percent acidity, total sugars, reducing sugars, non reducing sugars and ascorbic acid content are presented in Table 1. Post incubation at 37 ºC for 10 hrs it was observed that the free strains reduced the TSS of the juice from 12ºBx to 11.4ºBx and the encapsulated strains reduced it to 11.6ºBx. The pH reduced for both the samples with increase in per cent acidity showing inverse relationship between pH and acidity. Martin-Diana *et al.* (2003) also reported that adding probiotic starter culture caused decrease in pH value of the beverage at the same time titratable acidity was found to be increased. Total sugars for juice with free strains and encapsulated strains were found to be 6.1 and 6.4 respectively showing that free strains used up more sugars for the same time. The ascorbic content of both the samples decreased to 40 mg/100 ml which may have been due to heat treatment during juice pasteurization.

Table 1: Chemical Analysis of Probiotic Sweet Orange Juice

Properties	Before Addition of Strains	Juice with Free Strains	Juice with Encapsulated Strains
TSS (ºBx)	12	11.4	11.6
% Acidity	0.41	0.82	0.77
pH	3.9	3.51	3.68
Total Sugars (%)	8.36	6.1	6.4
Reducing Sugars (%)	1.8	1.5	1.7
Non Reducing Sugars (%)	6.6	4.6	4.9
Ascorbic Acid (mg/100 ml)	47.6	40	40

Note: * Each value is an average of 3 determinations.

Organolpetic Evaluation

The sensory evaluation was performed to examine the acceptance of probiotic juice by consumer against its taste, flavor and overall acceptability characteristics.Sensory scores of the freshly prepared probiotic juice samples are demonstrated in Table 2. According to the score, there was no significant difference in overall acceptability of the freshly prepared probiotic sweet orange juice samples containing free and encapsulated strains. However, considering the slightly higher score for juice with encapsulated strains, it can be concluded that the prevention of excess utilization of sugars by encapsulated strains which controlled the pH and per cent acidity production at optimum level may have resulted in better acceptability of the sample. The panellists also experienced improvement in taste of both the juice samples after probiotication.

Table 2: Mean Sensory Score of Freshly Prepared Probiotic Sweet Orange Juice Samples

Sample	Color	Taste	Flavor	Overall Acceptability
Control	8.6	8.3	8.1	8.2
Juice with free strains	8.3	8.4	8.5	8.4
Juice with encapsulated strains	8.5	8.6	8.5	8.5
SE	0.13176	0.06455	0.05528	0.02357
CD @ 1%	0.5443	0.26665	0.22835	0.09737

Note: * 9 Point Hedonic Scale.

Table 3 shows the overall acceptability of the juice containing free strains and encapsulated strains during a storage period of 4 weeks at 4 ºC. The overall acceptability score reduced with increase in storage period for both the samples, but it was observed that the overall acceptability score for juice containing encapsulated strains was always higher than juice with free stains throughout the storage. Thus, encapsulation clearly appeared to be effective in maintaining the sensory quality of the juice. This may be attributed to the inhibition of unfavourable deterioration reactions due to encapsulation. Similar results were reported in a study by King *et al.* (2007) where sensory scores of tomato juice containing microencapsulated probiotics were higher than that of free cells during refrigeration storage. Although, juice containing probiotic beads is a new concept, panel members compared the product to those of commercial juices containing juice sacs and thus found it acceptable.

Cell Viability of Juice with Free and Encapsulated Strains During Storage at 4 ºC

The cell viability of probiotic juice samples during storage is shown in Table 4. Post incubation at 37 ºC for 10 hrs, the cell count of the juice with free strains was found to be higher indicating more utilization of sugars and better growth while the cell count of the juice with encapsulated

Table 3: Sensory Score (Overall Acceptance) of Sweet Orange Juice Containing Free and Encapsulated Probiotic Strains During Storage (4 ºC)

Time in Weeks	Juice with Free Strains	Juice with Encapsulated Strains
0	8.4	8.5
1	8	8.3
2	7.7	8
3	7.3	7.9
4	7	7.5

Note: * 9 Point Hedonic Scale.

Table 4: Effect of Refrigerated Storage (4 °C) on the Viable Cell Viability of Free and Encapsulated Probiotic Strains in Sweet Orange Juice

Time in Weeks	Juice with Free Strains (cfu/ml)	Juice with Encapsulated Strains (cfu/ml)
0	$3.5x10^9$	$3.0x10^9$
1	$6.3x10^8$	$3.1x10^9$
2	$5.8x10^7$	$4.7x10^9$
3	$2.9x10^7$	$2.6x10^9$
4	$3.3x10^6$	$1.5x10^9$

strains was comparatively lower. However, during storage the viable count in the probiotic beads increased from an initial number of $3.0x10^9$ to $4.7x10^9$ during second week and further declined to $1.5x10^9$ in the fourth week. It was found that the viable cell count of encapsulated strains was maintained at 10^9 cfu/ml even after 4 weeks of storage. Free strains gradually lost their viability with increase in storage period and by 4th week the cell count decreased to 10^6 cfu/ml. These results indicates that encapsulation of strains increased probiotic survivability in the juice as encapsulated strains showed better survival than free strains after a refrigerated storage (4 °C) of 4 weeks. The encapsulation provided a protective barrier from the low pH and high acidity of the medium which would have otherwise affected the survival of the strains as it did in case of free strains. A study by King *et al.* (2007) showed that immobilized cells of probiotic lactic acid bacteria retained more during the cold storage period of ten weeks in fermented tomato juice compared with free cells because the immobilized cells were protected from oxygen, high concentrations of substrate and products, and unfavorable conditions such as low pH and high acidity. Ding and Shah (2008) also reported that probiotics encapsulated in orange juice and apple juice are more durable than the free cells.

CONCLUSION

From the study it can be concluded that sweet orange juice is a suitable substrate for the culture of probiotics as the strains grew and survived well when added to the juice and it was also found to be organoleptically acceptable. However, probiotic sweet orange juice prepared with encapsulated strains was found to be more acceptable than juice with free strains with increase in storage time. Also, encapsulated strains showed better survival with a cell count of 9 log cfu/ml even after 4 weeks of storage when compared to free strains which reduced to 6 log cfu/ml by the end of the 4th week indicating that encapsulation helps in maintaining cell viability effectively. Further, it was also observed that probiotication of the juice helped in improving the taste of the drink as experienced by the sensory panellists.

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APPENDIX

Figure 1: Overall Acceptability of Probiotic Sweet Orange Juice with Free and Encapsulated Strains During Storage (4 °C)

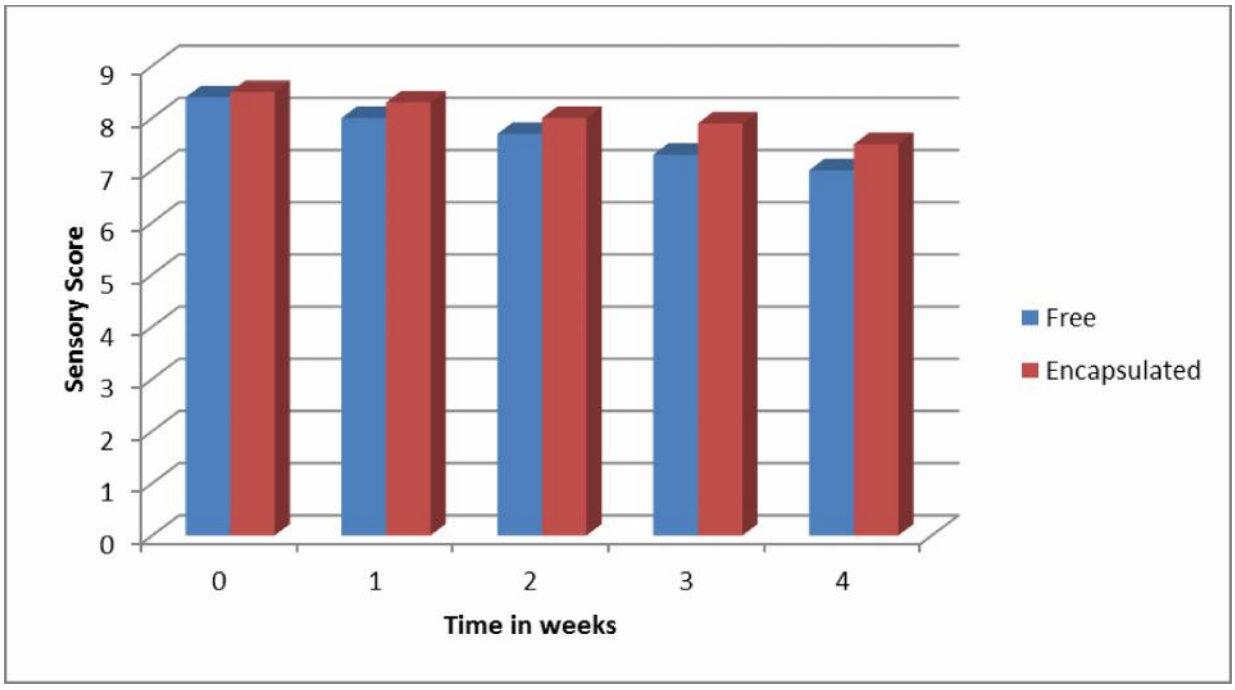


Figure 2: Cell Viability of Probiotic Sweet Orange Juice with Free and Encapsulated Strains During Storage (4 ºC)

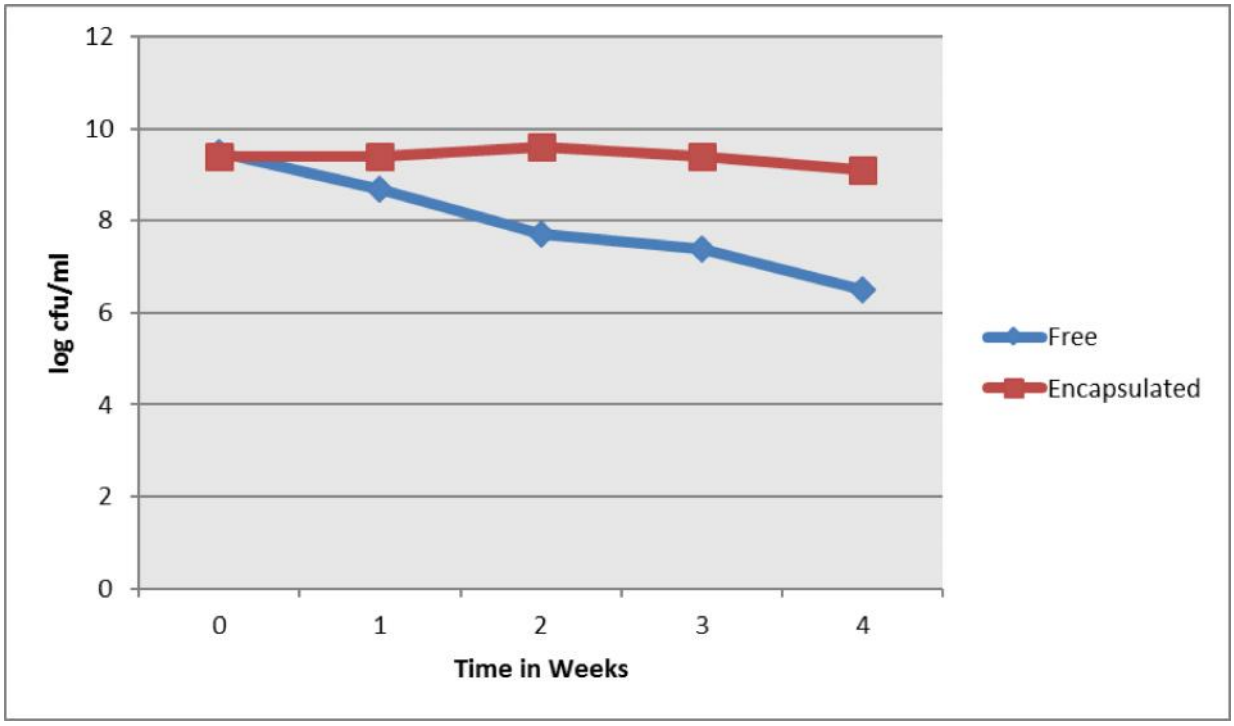
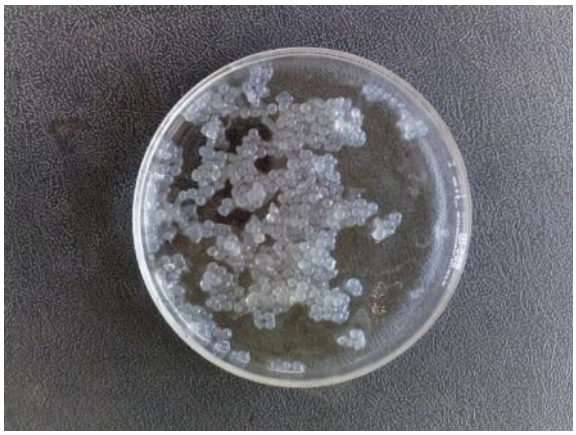


Figure 3: Lye Peeled Sweet Oranges

Figure 4: Probiotic Beads

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ANTI DIABETIC EFFECT OF CASSIA FISTULA AMONG SELECTED TYPE 2 DIABETES

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Diabetes has become a major global threat to human security and prosperity and overwhelms health systems completely. Medicinal herbs an alternative medicine system is extremely rich sources of type 2 diabetes remedies. *Cassia fistula* [family-*caesalpiniacea*] also known as Indian Laburnum is considered as a medicinal plant having hypoglycemic property. This study was carried out to find the antidiabetic action of the stem bark powder of *Cassia fistula* to selected type 2 diabetes. Twenty male subjects with type 2 diabetes who fulfilled the selection criteria were selected randomly and were equally grouped as experimental and control group. Three grams of *Cassia fistula* bark powder was supplemented to the experimental group for a period of 45 days with the approval of Institutional Human Ethical Committee. Biochemical tests such as fasting, postprandial blood sugar, HbA1c and urea and creatinine were estimated for both control and experimental groups and results were analysed statistically. The mean fasting and postprandial blood sugar level in the experimental group reduced from 153.50±11.157 mg to 120±9.07 mg and 235.50±32.524 mg to 206±30.67 mg respectively which was significant at 5% level and there was a reduction in the initial mean of 7.09±0.519% HbA1c in experimental group to 7.02±0.505% which was significant at $p<0.01$ level. The bark powder of Cassia fistula was found to be very effective in controlling blood sugar level of diabetes and proved its importance as a valuable medicinal plant.

Keywords: Type 2 diabetes, Bark, Anti diabetic, Blood sugar, Medicinal plant

INTRODUCTION

Diabetes Mellitus is a disorder of metabolism of carbohydrate, protein and fat due to absolute or relative deficiency of insulin secretion and with varying degree of insulin resistance (Alagapan, 2000). The recent International Diabetes Federation (IDF, 2011) has pegged 366 million people to suffer from diabetes which is expected to reach a staggering 552 million in 2030 despite of several effective preventive efforts and actions.

Diabetes leads to significantly reduced quality of life and life expectancy due to life-threatening co-morbidities and complications (Peter, 2013). Wlid *et al.* (2004) indicated that the population growth, ageing, urbanization and increasing prevalence of obesity and less physical activity of people has lead to the increased incidence of diabetes. Traditional medicinal plants and herbs possess no or fewer side effects, are easily available and are relatively of low cost (Valiathan, 1998; and Vasudevan *et al.*, 2009).

Cassia fistula (Caesalpiniaceae), known as "Golden Shower", "Indian laburnum" is a medicinal plant of immense importance (Asolkar, 1992) and has been referred to as "Aragvadha" or "Disease Killer" in ayurveda (www. Ayurvedichomeremedies.com, 2013). All parts of the plant have medicinal value and have a high therapeutic value and it exerts an antipyretic and analgesic effect (Patel *et al.*, 1965).

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