**DEVELOPMENT OF LOW COST PROBIOTIC CHIKKIES AS FOOD SUPPLEMENTS FOR THE BENEFIT OF HUMAN HEALTH****¹Chaitanya Tottalla,²Hannah Jessie Francis.T and ³Shiva Prakash M**¹MSc Student in Clinical Nutrition and Dietetics, ²Research Guide & Lecturer and ³Former Scientist^{1&2}Department of Nutrition, St. Ann's College for women and ³National Institute of Nutrition (ICMR)^{1&2}Mehdipatnam and ³Jamai-Osmania P.O., Tarnaka

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ABSTRACT

Background: Micronutrient deficiencies are one of the most frequent deficiencies around the world. Low intake of micronutrients and their poor bioavailability in humans causes various micronutrient deficiencies. Therefore, development of probiotic based food supplements will be helpful for efficient absorption and bioavailability of nutrients as there are number of evidences reported from developing countries particularly from India. The present study was aimed to incorporate probiotic micro flora into the food products.

Objectives: Development of low-cost probiotic-based food product viz., probiotic chikkies and assess sensory evaluation along with viability upon storage.

Methodology: Three different Probiotic chikkies i.e., groundnut, sesame were prepared by adding *Lactobacillus bulgaricus* (delbrukisubsp) (NIN Lb-01) and *Streptococcus thermophilus* (NIN St-05) isolated from commercially available curds Hyderabad were tested for confirmation of the species and for purity. The Probiotic strains were revived in respective culture medium and performed for Gram's Staining. The species were identified various bio chemical reactions using API (Analylab Product Inclusive) system as well as through Gram's staining. These probiotics were lyophilized and were added during the process of making chikkies. Sensory evaluation was carried out by user's 5-point hedonic scale in human volunteers. A questionnaire was specially designed for obtaining their knowledge on probiotics. Simultaneous, viability was carried out at different time points upon storage at room temperature. The data obtained was compiled on excel and subjected and statistically analysis using 20.0.

Results: The present study indicated that the overall acceptability for probiotic chikkies is similar to non-probiotic chikkies. Further the viability of probiotics retained up to a period of one month which was sufficient for normal physiology in humans.

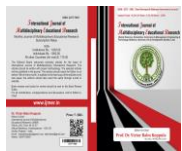
Conclusion: From the study it can be concluded that the probiotic-based food supplements may be helpful in the nutrient absorption particularly micronutrients, which would help in management of human health.

Keywords: Micronutrient deficiencies, Probiotics, Lactic acid bacteria, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, Peanuts, Sesame, Chikkies, Gram staining, Viability.

Introduction

India with divergent food habits is having a number of traditional foods, including sweet products. Chikki is one of the popular Indian traditional sweet snacks. Chikki is mainly prepared using jaggery as sweetener and roasted peanuts (*Arachis hypogaea*) (1) and contains protein, minerals and vitamins and is a potent source of iron and copper (2). Groundnuts (*Arachis hypogaea*) or peanut is a legume which is widely grown as one of food crops. Groundnut seed contains 44%–56% oil and 22%–30% protein on a dry basis, and is a rich source of minerals (P, Ca, Mg, and K) and vitamins (E, K, and B group) (3). Usually, other ingredients such as puffed Bengal gram, sesame, puffed rice, beaten rice and copra (desiccated coconut) are used and some chikkies are made using a combination of these ingredients.

Probiotics are generally considered as safe organisms where they confer health benefits to the host when consumed in adequate levels. The contribution of lactic acid bacteria (LAB) in designing and developing functional non-dairy foods presents interesting prospects and has begun to be exploited for redesigning traditional foods with enhanced benefits. The many benefits of LAB range from enhancing the shelf life and safety of foods, improving food textures, and contribute to the nutritional value of food products through removal or reduction of anti-nutrients without changing of pleasant sensory profile. Furthermore, LAB have been exploited for generating bioactive molecules such as γ -amino butyric acid (GABA) in both dairy and non-dairy products (4). Use of different probiotic blends in combination have several health benefits. Thus, a number of studies have shown that lactobacilli, not only constitute an integral part of the host's gastrointestinal micro ecology (5), but also play an important role in the host's immunoprotective system by increasing specific and non-specific immune mechanisms (6) and growth status of malnourished young children (7). *Lactobacillus plantarum* 299 is a probiotic strain hypothesized to have a positive outcome for iron absorption and also it has been shown that lactic-acid fermented vegetables and cereals can increase the iron absorption. (8) Hence the present study is aimed to evaluate the sensory characteristics and viability of the Probiotic chikkies developed by incorporating Probiotic microflora viz *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.



Methodology

Testing the purity of Probiotics

The probiotics powders obtained from Unique Biotech, Hyderabad were tested for confirmation of the species and for purity. The species were identified using various biochemical reactions using API (Analylab Product Inclusive) system as well as through Gram’s staining.

Preparation of Probiotic Chikkies

Two types of chikkies have been prepared in the study. Peanuts (*Arachis hypogaea*), Sesame seeds (*Sesamum indicum*) and jaggery were procured from local market. Peanut seeds were roasted to golden brown colour (120–130°C), dehusked, de-germed and crushed into small bits of about 2.8 mm. Sesame seeds were roasted until they change colour for about 4 to 5 minutes and allowed to cool.

Preparation of chikki

Chikki was prepared by taking jaggery and peanuts in equal proportions. Jaggery was crushed and made into syrup with addition of water and warming and filtered through a nylon mesh of ~ 30 mesh to remove extraneous matter. The clear jaggery syrup was heated until the temperature reached 145 °C and immediately pre-weighed, roasted and dehusked peanuts were added and mixed thoroughly till the nuts get coated with jaggery syrup. The mixture was kept aside till it reaches to a lukewarm temperature (55⁰-60⁰ C) and about 10x10⁹ cfu/ml (*Lactobacillus* + *Streptococcus thermophilus*). The mixture was then transferred on to a greased wooden board or clean platform. The product was then spread uniformly by rolling it with the help of a roller. Vertical and horizontal lines were marked with a cutter to make individual slabs of about 15-20gms each and then cooled to room temperature (27 ± 2 °C) and were packed in airtight container. Simultaneously Sesame chikki has been prepared by addition of toasted and sieved sesame seeds replacing peanuts in chikki preparation. Chikkies without adding probiotics were also prepared to be served as controls.

Sensory evaluation of product developed

Prepared Probiotic chikkies were subjected for sensory evaluation by ten semi- trained panelists. The organoleptic properties i.e., Aroma, Appearance, Texture, Taste, Acidity, Mouthfeel and Overall acceptability of the chikkies developed were evaluated by trained panel of 10 member using five – point hedonic scale scoring system scale (1= dislike extremely, 2= dislike moderately, 3= neither dislike nor like, 4= like moderately and 5= like extremely). The evaluation was carried out at room temperature in the metabolic kitchen located at NIN-Hyderabad. Each panelist scored samples independently and recorded the scores on the sheets provided. Panelists were served water and unsalted crackers to clean their mouths before tasting each sample.

Viability of Probiotics

The prepared products were tested for the viability within three hours of preparation as well as in stored samples after 15 and 30 days. The microbiological method i.e., Spread plate method (9) used for bacterial count on MRS plate is described below.

One gram of sample was transferred into a test tube containing 9 ml of saline. It was mixed well by shaking for 2 min. One (1) ml from this was taken and diluted further through a series of test tubes containing 9 ml of sterile normal saline blank solution by an appropriate decimal dilution method. Recommended final dilution was up to 10⁸ -10⁹. Then, from the final diluted tube, take 100µl and pour into the MRS agar medium Petri dishes and spread into medium by clockwise and anticlockwise via spread plate technique. The plates were then incubated at 37°C for 24 - 48 hours and the number of colonies in each plates was counted in colony counter machine. The isolated colonies were sub cultured and subjected for gram staining for identification of species.

Lactobacillus and *Streptococcus* appeared as large, white colonies in or on the surface of the medium. The bacterial colonies were counted using colony counting machine and expressed as colony forming units (CFU) per gram or ml of sample, taking into account the applicable factor. The average number of colonies, multiplied by the dilution number (dilution factor) and divided by quantity of sample on weight basis and expressed as colony forming units/gram and statistical analysis was carried out using computer software SPSS version 20.0

$$\text{Number of colony forming units} = \frac{\text{Total number of colonies}}{\text{Amount of sample plated X Dilution factor}}$$

(CFU) per g of the sample

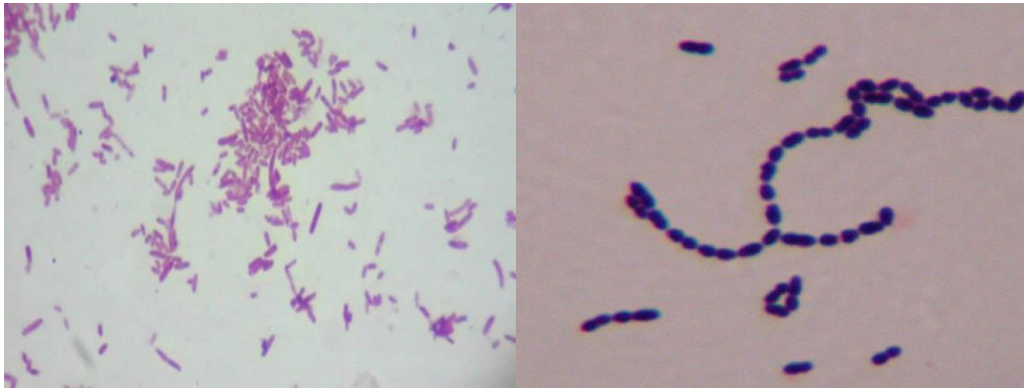


Figure 1&2: Gram's staining of Lactobacillus & Streptococcus thermophilus



Figure 3&4: Probiotic Groundnut & Sesame chikkies

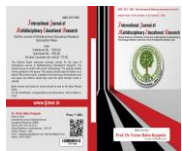


Figure 5 & 6: Viability of Probiotic bacteria from the products developed.

Results and Discussion

The overall acceptability was similar for both probiotic groundnut chikkies(74%) and non-probiotic groundnut chikkies, however it was not statistically significant. The non-probiotic groundnut and probiotic groundnut chikkies have the similar score, which ranged from 3.1 to 4.1 in 5point hedonic scale. There was no significant difference in the sensory attributes and overall acceptability for probiotic sesame chikkies was 76%.

The non-probiotic sesame chikkies and probiotic sesame chikkies have the similar score, which ranged from 3.2 to 4.2 in 5-point hedonic scale. This indicates addition of probiotics does not affect the sensory attributes.



Mean scores of non-probiotics and probiotic chickies were not significantly different from each other in sensory attributes i.e., aroma, appearance, texture, mouth feel and overall acceptability as shown in the table 4&5. Therefore, it was that no difference in sensory qualities were found in both probiotic groundnut and probiotic sesame chickies when compared with non-probiotic products. They were very similar to control products in all organoleptic qualities.

From the table 6&7 it was observed that the viability of probiotic microorganisms has been reduced from 8.44×10^6 cfu/gm to 5.60×10^6 during the storage of 15 days, and further reduced to 1.88×10^6 cfu/gm on 30th day of storage. The survival rate decreased to 97.42 % on 15th day and to 92.71% on 30th day.

The viability of probiotic microorganisms in sesame chicki were reduced from 10.24×10^6 cfu/ gm to 9.76×10^6 cfu/gm on 15th day of storage, and further reduced to 1.88×10^6 cfu/ gm on 30th day of storage. The survival rate decreased to 99.7% on 15th day and to 89.4 % on 30th day. This shows that climate and environment at room temperature were favouring the viability. However, the difference of viable counts from 0 day to 15 day and 15 days to 30 days were found to be not significant at $P < 0.05$.

Table 1: Acceptability of Probiotic chickies on the day of preparation (Mean \pm S.E) and percentages within brackets

Attributes	Groundnut Chickies		Sesame Chickies	
	With Probiotics	Without Probiotics	With Probiotics	Without Probiotics
Aroma	3.8 \pm 1.13 (76%)	3.9 \pm 1.13(78%)	3.8 \pm 0.91(76%)	3.6 \pm 0.84(72%)
Appearance	3.8 \pm 0.99(84%)	3.1 \pm 0.99(78%)	3.7 \pm 0.82(74%)	3.7 \pm 0.82(74%)
Texture	4.2 \pm 0.78(76%)	3.9 \pm 0.87(78%)	3.2 \pm 0.78(64%)	3.3 \pm 0.94(66%)
Taste	3.8 \pm 0.63(76%)	3.9 \pm 1.10(76%)	4.1 \pm 0.56(82%)	3.6 \pm 0.51(72%)
Mouthfeel	3.8 \pm 0.91(76%)	3.8 \pm 1.13(76%)	3.8 \pm 0.91(76%)	3.5 \pm 0.84(70%)
Overall Acceptability	3.7 \pm 0.8(74%)	4.1 \pm 0.87(82%)	3.8 \pm 0.91(76%)	3.8 \pm 0.78(76%)

- Indicates no differences in the sensory evaluation between the with and without Probiotics for sensory evaluation in the developed Chickies

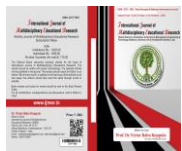
Table 2: Viability of Probiotics during different periods of storage.

Product	0 Day	15 th Day	30 th Day
Probiotic Groundnut chickies	8.44×10^6 cfu ^a (log 6.926342)	5.60×10^6 cfu ^b (log 6.748188)	1.88×10^6 cfu ^c (log 6.421604)
Probiotic Sesame chickies	10.24×10^6 cfu ^a (log 7.0103)	9.76×10^6 cfu ^b (log 6.98945)	1.88×10^6 cfu ^c (log 6.274158)

^{a,b,c} Differences in the superscripts indicates statistical significance between different time points for decline viability in cfu/gms

Table 3: Viability (%) of probiotics in different periods of storage.

Product	0 Day	15 th Day	30 th Day
Probiotic Groundnut chickies	100%	97%	92%
Probiotic Sesame chickies	100%	99.7%	89.4%



Discussion

Overall, it was observed that probiotic incorporated groundnut chikkies and sesame chikkies were favorable to retain the viability of the *L. bulgaricus* and *S. thermophilus*. These live microorganisms are sufficient to have beneficial effect upon consumption i.e., to have probiotic effect.

The developed probiotic chikkie contained 1.024×10^7 cfu/ gm in the chikki, which was found to be reduced to to 9.76×10^6 cfu/gm at 15th day. Further the viable count was reduced to 1.88×10^6 cfu / gm on 30th day of storage. The survival rate decreased to 99.7% on 15th day and to 89.4 % on 30th day. This shows that climate and environment at room temperature were favoring the viability. However, the difference of viable counts from 0 day to 15 day and 15 days to 30 days were found to be not significant at $P < 0.05$. In support of these findings a stability study, done by (10) on *L. sporogenes*, incubated at 45 ± 1 °C for 90 days demonstrated that the spores maintained their viability and their level of residual activity was close to 100 %.

Overall, it was observed that probiotic incorporated groundnut chikkies and sesame chikkies were favorable to retain the viability of the *L. bulgaricus* and *S. thermophilus*. These live microorganisms are sufficient to have beneficial effect upon consumption i.e., to have probiotic effect. Probiotic lactobacilli may help correct malabsorption of trace minerals, found particularly in those with diet high in phytate content from whole grains, nuts, and legumes (11)

In a study it was found that the iron absorption was improved after supplementation that may be related to the colonisation of Lp299v in the intestine. A mannose adhesion-encoding gene in *L. plantarum* has been identified(12), and it has been shown that Lp299v can adhere to the intestinal epithelium via a mannose-binding mechanism (13) (14) showed that Lp299v can also adhere to mucin that covers the epithelium in the intestine, and in vitro trials indicate that Lp299v increases the mucin excretion(15). Mucin may be involved in iron absorption (16) showed that mucins can bind iron and that these mucin-iron complexes prevent precipitation of the iron(17).

In a study done by (18) it was shown that the lactic acid fermented foods can increase iron absorption in humans, possibly by lowering pH, activating phytases, producing organic acids or by the viable lactic acid bacteria. Another possible mechanism underlying the observed results could be an increase in colonic iron absorption due to a decrease in colonic pH, thereby reducing ferric iron into highly absorbable ferrous iron as a result of lactobacilli growth. In a cell-line study by (19) lactic acid fermentation by *Lactobacillus* enhanced Caco-2 iron uptake from carrot juice. As soluble ferrous iron was increased about 16-fold by lactic acid fermentation, and about one-third of the ferrous iron remained soluble after in vitro digestion (about 4- to 5-fold higher than in fresh juice), the authors concluded that enhanced iron uptake was a result of the increased level of soluble ferrous iron.

Conclusion

From this study it can be concluded that the Sensory Evaluation showed highest acceptability of the probiotic chikkies, therefore it can be recommended for human consumption. Probiotic incorporated chikkies sustained viability after exposure to a temperature of 50- 60°C. The viability of a probiotics didn't decline in the developed product and product retained sufficient number of viable probiotics. Therefore, the developed probiotic product is safe and can be stored and supplemented for a period of one month. The above product can also be incorporated with various probiotic combinations to study their benefits. However, these findings can be further strengthened by supplementation of the developed products on vulnerable population.

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