Formulation of Non-Dairy Synbiotic Drink from Chickpea Supplemented with Chicory Root Powder and Assessment of Its Market Potential

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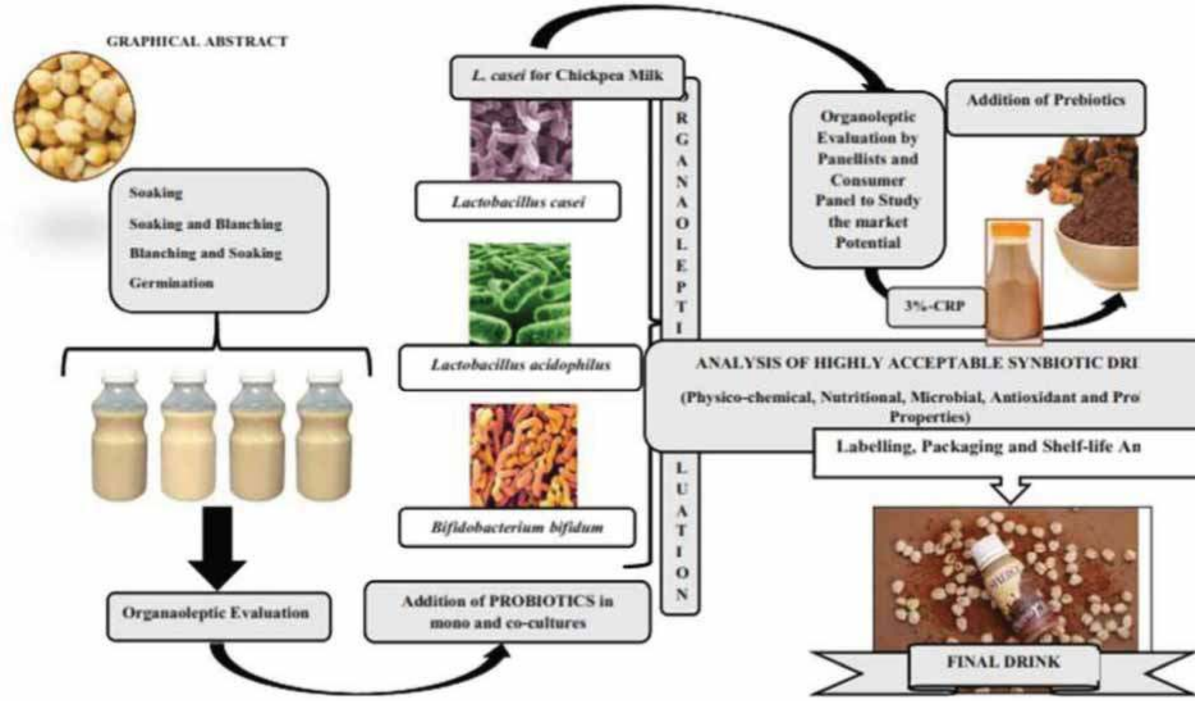
ABSTRACT

Formulation of Non-Dairy Synbiotic Drink from Chickpea Supplemented with Chicory Root Powder and Assessment of Its Market Potential. Working in a multicultural dietary habit, the demands for a wide range of non-dairy functional foods, that are just as effective as dairy products, have increased globally. These can be consumed amid usual diet and potentially prevent diseases and promote optimum health. The Functional beverage industry includes energy drinks, fortified juices, sports drinks, dairy and dairy substitute drinks, enhanced water, ready-to-drink tea and coffee. Minerals, vitamins, herbs, amino acids, antioxidants and microorganisms in functional beverages greatly facilitate different physiological processes such as the immune system, heart rate, digestive health, and weight management. Because of the rising prevalence of lactose intolerance and the rising number of people following vegan, macrobiotic, flexitarian lifestyles, the demand for non-dairy beverages has surged. The options for non-dairy substitutes can be cereals, legumes and nuts. So, the present study was carried out to prepare legume milk from Chickpea by various processing methods and short-listing the most acceptable milk for fermentation by mono and mixed cultures of different probiotic bacteria. Organoleptically, the most acceptable probiotic extract was enriched with prebiotic food, chicory root powder, rich in inulin, in different proportions. The enriched drinks were organoleptically tested by a semi-trained and a consumer panel to short-list the highly acceptable variation of the developed drink, and predict its market potential. Furthermore, the highly acceptable variation was analyzed for its physico-chemical, nutritional, antioxidant, probiotic and microbial properties. The drink was packaged, labeled and stored at 4°C/60 days for shelf-life analysis. The shelf-life study indicated that the developed synbiotic drink was acceptable till 40 days of storage, both organoleptically and microbiologically. The cost of the packaged bottle reported that it is economical and can be brought and consumed on regular basis.

Keywords: chickpea, enriched, inulin, organoleptic, shelf-life, synbiotics



I INTRODUCTION

Substantial changes have been observed in the Indian food and beverage industry in recent years. Recently, functional foods are occupying an important place among both academic and industrial trends as they improve the quality of dietary intake by providing the required nutritional components for the human organism [1]. On the other hand, beverages are one of the most accessible foods which could provide nutrients such as vitamins, mineral substances, antioxidants, organic acids and other active biological substances for the body [2]. In this regard, there are huge demands for the development of new non-dairy based probiotic beverages to the functional food market by using controlled fermentation by probiotics and incorporation of prebiotics.

Enriched, enhanced, fortified and flavoured functional foods, when taken as a part of regular diet provide lots of health benefits, but this claim doesn't withstand with all the functional food available in the market. The reason is the disparity in functional properties of the functional foods, such as soy proteins, legumes, dairy-based foods and the intangible resources. Though, consumers do select among variety of food spectrum having functional components either intrinsically (like soy protein or cranberries) or via fortification (like folate-fortified foods) but India still have reasons, scope, space and market for development of functional foods. Foods for Specified Health Uses (FOSHU) are also attracting consumers' attention [3].

Pro- and Synbiotic food products can be reinvented by applying modern technologies for mass production to meet the consumers rising demand for healthy drinks. Present day consumers prefer foods that provide optimum health and aids in preventing diseases. Furthermore, such functional foods should become the part of current lifestyle patterns i.e., vegan, gluten-free, flexitarian, macrobiotic etc., providing utility convenience, enhanced flavours and an acceptable price-value ratio. Such foods constitute current and future trends in the evolution of the food development cycle.

The current research is about the development of non-dairy Chickpea (ChP) based synbiotic drink, by combining the potential health benefits of the probiotics, with their ability to thrive in the legume matrix, enriched with prebiotics, resulting in a nutritionally healthy and desirable drinks for the consumers. The present study was planned aiming at processing Chickpea (Cicer arietinum)- Cultivar CSJK 114 for extracting the best acceptable milk and inoculating it with LAB i.e., Lactobacillus casei, Lactobacillus acidophilus and Bifidobacterium bifidum, in mono and co-cultures. Furthermore, the most acceptable probiotic extracts were supplemented with the selected prebiotic i.e., Chicory Root Powder (CRP) in different variations. The developed synbiotic drinks were, then, subjected to consumer acceptability and the highly acceptable drink was labelled, packed and analyzed for shelf life. Finally, the cost analysis was done keeping in mind its commercial availability. Therefore, the current investigation was aimed to produce a novel legume-based synbiotic drink fermented by Lactobacillus casei, with appropriate quality attributes, and shelf stability.

II MATERIALS AND METHODS

This research was carried out in the Research and Microbiology Laboratory of Department of Home Science, IIS (Deemed to be University), Jaipur. Organoleptic analysis was done by shortlisting the semi-trained panellists (n=15), comprising of the faculty members and research scholars of the University. For the statistical analysis, SPSS (28.0v) software was used and random sampling method was employed to conduct the study of the market potential and overall acceptability of the drink.

(a) Extraction of Legume Milk - The legume was procured from Rajasthan Agricultural Research Institute, Jaipur and were carefully examined for any broken or distorted seeds, as well as any extraneous material such as stones. The cosmetically inspected beans were kept at room temperature in food-grade, air-tight plastic boxes before further handling. The cleaned, preserved legumes were hand-washed thoroughly with fresh water before undergoing through four distinct milk extraction methods i.e., soaking, soaking and blanching, blanching and soaking; and germination. After processing, the excess water was drained out and the legumes were dehulled and wet-milled continuously for 5-10 minutes, with warm distilled water (40°C-50°C), by using a food processor (PHILIPS HL1659/00) and legume slurries were prepared. To, 250 gm of legumes, 750mL of distilled water was added to obtain a filtrate of milk-like consistency. The slurries were then filtered through double-layered cheesecloth to remove insoluble fibre and suspended soluble fibre. The product obtained were the legume milks [4]. The by-products, i.e., insoluble fibre and suspended soluble fibre present in the legume slurry, were further utilised to make other edible products like Hummus. The legume milk was sterilized at 108-120°C for 10-15 minutes. The milks obtained from both the legumes were stored in clean 500mL transparent glass bottles. The glass bottles, plugged with cotton and wrapped with aluminium foil, were suspended in boiling water for 10 minutes. The crowns of the bottles were also sterilized using the same technique. The prepared milks were crowned, labelled and pasteurized at 85°C for 15 minutes, cooled and stored for organoleptic evaluation under ambient conditions narrowing down the picking of the most acceptable milk for finished product formulation. The organoleptic evaluation was carried out by employing 5 Point and Hedonic Rating scales, after training the panellists by using Triangle test [5].

(b) Development of probiotic extracts by using mono and co-cultures of the selected bacteria - The lyophilized bacteria i.e., Lactobacillus casei NDRI strain RTS, Lactobacillus acidophilus NDRI HI 39 and Bifidobacterium bifidum NCDO 2715 were procured from National Dairy Research Institute, Karnal. MRS agar culture medium, MacConkey agar, potato dextrose agar sodium chloride and hydrochloric acid were supplied by Hi-Media Pvt Ltd., Mumbai. The bacteria were revived and cross streaked to study the possibility of any antagonistic interactions between the selected LAB, to ensure proper co-culturing [6]. The most acceptable legume milks were taken as a substrate for fermentation by the selected bacteria, in mono and co-culture forms, and the development of probiotic extracts were carried out by using serial dilutions and pour plate technique [7]. Further, the organoleptic evaluation was done to shortlist the most acceptable probiotic extracts.

(c) Development of synbiotic drinks and study of its' consumer acceptability and market potential - The most acceptable probiotic extracts were shortlisted on the basis of the results obtained after the organoleptic evaluation of all the five variants made. The variants obtained were enriched with prebiotic CRP to balance the gut microbiota. For the assurance of a homogenous drink, food stabilizer was added and sugar was also added to enhance the taste and favour. To see the acceptability of the synbiotic drinks among the consumers and its potential in the market, the drinks were assessed by a consumer panel of 100 members. A Performa was developed; consisting questions regarding the organoleptic parameters of the drinks, frequency of consumption, recommendation of the drinks to others, probable cost of the drinks and the overall experience of the consumers; and administered to identify the highly acceptable drinks.

(d) Analysis of physico-chemical, nutritional, microbial, antioxidant and probiotic properties of the highly acceptable drink

(i) Physico-Chemical Analysis - The physico-chemical analysis of the synbiotic drinks was done by estimating the pH, viscosity, peroxide value and acidity using standard methods. Digital pH metre (Elico LI-127) was used to estimate the pH and Brookfield Viscometer was used for measuring the viscosity of the developed drinks (Model LV DV-E 230; Middleboro, Massachusetts, USA). Peroxide Value was determined by the method given by Pearson [8] and the acidity was determined by using the protocol suggested by Ranganna [9].

(ii) Nutritional Analysis - The highly acceptable drinks were analyzed for moisture, crude fat, crude fibre, crude protein, total ash, carbohydrates and total calorie content by using the standard protocols [10] and chemicals of analytical grade.

(iii) DPPH radical scavenging activity - Radical scavenging activity of methanolic carrot extracts against stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) was measured using a slightly modified method of Sharma and Bhat [11].

(iv) Probiotic Properties - When a probiotic-based dietary supplement is ingested, it has to face the harsh conditions of the stomach and survive under the extremely acidic conditions, where the pH generally ranges from 2-3. Again, a transit time of about 4 hours in the stomach is another challenge faced by the probiotic organisms. Thus, survivability in acidic conditions is a test the probiotics has to pass. After surviving the acidic conditions of the stomach, the probiotic organism has to face the bile in the duodenum. Survival in the bile atmosphere depends on the concentration of bile and the exposure time. Bile acid shows antibacterial activity and thus the probiotic organism has to show bile resistance in order to move down further in the GI tract. In vitro acid and bile resistance properties of the isolated probiotics were thus studied to prove the efficacy of the functional drink developed in this study [12,13].

(e) Development of food label, aseptic bottling, shelf-life and cost analysis of the packaged drinks - The development of an appropriate food label and packaging of the highly acceptable drinks was done, keeping in mind the commercial availability of the developed synbiotic drinks, the shelf-life and cost analysis was also performed. As Polyethylene Terephthalate (PET) is suitable for packaging drinks because of its light weight, glass-like appearance, shatter resistant and rigidity, so PET bottles were procured from Periwal Plastic Industries, Jaipur. The packaged bottles were stored under refrigeration throughout the period of the study. Appropriate food labels were developed and designed by using CorelDRAW. The food labels were developed for the highly acceptable drinks in accordance to the Food Safety and Standards (Packaging and Labelling) Regulations, 2011. The highly acceptable synbiotic drink was then studied for its shelf stability for 60 days, at an interval of 10 days, for its organoleptic, physico-chemical and microbial parameters. Finally, the cost calculation was done.

III RESULTS AND DISCUSSION

The present study was carried out to investigate the possibility of producing novel synbiotic beverage by incorporating probiotic microorganisms into legume matrix and supplementing it with prebiotic, CRP.

(a) Assessment of organoleptic properties of ChP milk obtained after employing various processing techniques-The results of the organoleptic evaluation of the ChP milk after the application of various processing techniques, in comparison to the commercially available unsweetened soy milk, SOFIT (CONTROL) was carried out and the results were tabulated in Table No. 1.

Table 1

Mean Scores of the Organoleptic Parameters of the ChP Milks obtained by Different Processing Techniques

Processing method / Parameter	Control	Soaking	Soaking and Blanching	Blanching and Soaking	Germination	F value	p value
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Appearance	8.67^{a} ±0.48	7.93^{b} ±0.70	**8.73^{a} ±0.45**	7.27^{c} ±0.59	6.07^{d} ±0.79	3.23*	0.023
Colour	8.20^{a} ±0.41	7.33^{b} ±0.49	**8.33^{a} ±0.49**	5.87^{d} ±0.83	6.60^{c} ±0.51	2.94*	0.033
Texture	8.60^{a} ±0.51	7.80^{bc} ±0.86	**8.06^{ab} ±0.70**	7.20^{c} ±0.87	6.40^{d} ±0.51	21.47***	0.000
Taste	8.06^{a} ±0.46	7.47^{c} ±0.52	**8.00^{ab} ±0.76**	7.40^{bc} ±0.51	7.43^{c} ±0.74	71.63***	0.000
After taste	8.20^{ab} ±0.56	7.53^{b} ±0.74	**8.27^{a} ±0.70**	6.93^{c} ±0.59	6.00^{d} ±0.84	2.33*	0.032
OA	8.33^{ab} ±0.49	7.86^{b} ±0.83	**8.53^{a} ±0.52**	7.13^{c} ±0.74	6.27^{d} ±0.59	3.90**	0.004

(i) **Mean values represent the average of 15 determinants**

(ii) **a, b, c, d - The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level**

(iii) **Level of significance in increasing order – (*$p<0.05$, **$p<0.01$, ***$p<0.001$)**

Amongst all the legume processing techniques, the milk obtained after soaking and then blanching was found to be the most acceptable by the panel members. There was no significant difference between the organoleptic parameters of control and the milk obtained after soaking and then blanching. The overall acceptability scores for the milks of ChP were highest after soaking and blanching, which were found to be 8.53. The milk produced after germination scored the least on all the organoleptic parameters. The incorporation of the by-products into Hummus was acceptable and successful. The main goal of this phase was to develop legume milk from ChP focusing on the best technological options to obtain a high-yield extract with reduced "beany" flavour, with the least possible discharge of by-products. As a result, a series of processing methods were employed to improve the organoleptic characteristics and beverage stability. The processing steps used were soaking, blanching and germination. To serve as a guide for the preferred consumers' taste, texture, and aftertaste, the obtained milks were compared to the commercial non-dairy legume-based beverage, SOFIT. A major limiting factor in the organoleptic acceptability of these beverages based on legumes, is their characteristic "beany" flavour which is linked to endogenous lipoxygenases that oxidise unsaturated fatty acids in oil-rich pulses like soy and peanuts (over 20% fat), therefore it's likely to be less evident in oil-poor pulses like peas, lupins, or chickpeas (1.5 percent to 5 percent fat). Heat inactivation is a potential approach for neutralizing these off flavours from legume-based beverages [14]. Legume seed bioactives (e.g., phytate, protein inhibitors, phenolics, tannins, lectins and saponins) might affect a consumer's health in a variety of ways [15,16]. ChP do not present much of these bioactives, being the phytic acid the relevant factor. It has been established that soaking pulse grains lowers polyphenols and removes any residual alkaloids; minimizes cooking time, enhances starch gelatinization (e.g., in Chickpeas) and boosts protein bioavailability and peeling [17,18]. Blanching and germination, on the other hand, can be a helpful strategy to minimize anti-nutritional variables since it diminishes the bitterness and "beany" flavour of grains, due to the presence of phytates [19,20]. Blanching with hot water was employed to inactivate enzymes like lipoxygenase and trypsin inhibitors for improvement of the flavour and nutritional value of the non-dairy milk alternatives [21]. It has been reported that blanching is effective in diminishing the beany, grassy, bitter and rancid flavour; it also prevents suspension instability and chalkiness in non-dairy milks [20]. Blanching with hot water (85-100°C for 2-5 minutes) has been commonly used for skin removal of raw materials and overcoming off flavours in non-dairy milk alternatives. Blanching inactivates enzymes, reduces possible microbial contamination, and aids in deskinning in processing by wet methods [22].

New drinks from diverse pulses (i.e., chickpea, lupin and pea) were produced by Lopes et al. [4] utilizing technologies that allow for the inclusion of a high level of seed components with minimum or no by-product discharge. The researchers attempted to test different processing steps sequentially in order to optimize the sensorial features and stability of the beverages, considering the current commercial non-dairy beverage trend. Results of the sensory quality showed that ChP beverage prepared by using cooking water had the best taste and overall acceptability.

As far as the usage of the by-products of ChP was concerned, the infilterate was employed in the making of Hummus. Though, it can be used in many other ways as pasta and pizza sauces, can also be dried and powdered. The powder can be used in various recipes like chapatti, pizza, dhokla, cookies, breads etc., owing to its high nutritional value and functional properties. Okara is a by-product obtained from soy milk extraction. A novel-type of food product was gluten free flour which was produced by drying the okara and incorporating it with other ingredients for baking purposes [23]. Gluten-free cookies have been developed using okara and commercial manioc flour [24,25]. Similarly, the current study also concludes that there is a wide scope for the usages of the by-products of ChP, which can be dehydrated and used for many more recipe formulations and can be used for value addition in biscuits, chapatti, cakes, cheela, cookies, pancakes, muffins etc.

(b) Development of probiotic extracts by using mono and co-cultures of the selected bacteria

(i) **Cross streaking of selected bacteria -** Cross streaking is a rapid qualitative isolation method. This method was used in the current study for testing the inhibitory activities of the three selected strains of probiotics i.e., Lactobacillus casei NDRI strain RTS, Lactobacillus acidophilus NDRI HI 39 and Bifidobacterium bifidum NCDO 2715 against each other. This test was specifically performed as the probiotic extracts were not only prepared by using single strains, but by using consortia (co-cultures). The antagonistic effect of the selected strains was studied against each other by cross streaking and the results depicted that there was no antagonistic activity seen amongst the three probiotic bacteria (Fig No. 1). The microbial interaction was analysed by observing the size of the inhibition zone. The inhibition zones between the selected probiotic strains were observed for two days. Table No. 2 shows the antagonistic effect of selected probiotic strains against each other as observed by agar well diffusion method.

Table 2
Antagonistic Effect of the Selected Probiotic Strains against each other by Agar Well Diffusion Method

S.No.	Probiotic strain	Diameter zone of inhibition (in mm)	
		Day 1	Day 2
1	Lactobacillus casei NDRI strain RTS	0	1
2	Lactobacillus acidophilus NDRI HI 39	0	1
3	Bifidobacterium bifidum NCDO 2715	1	3

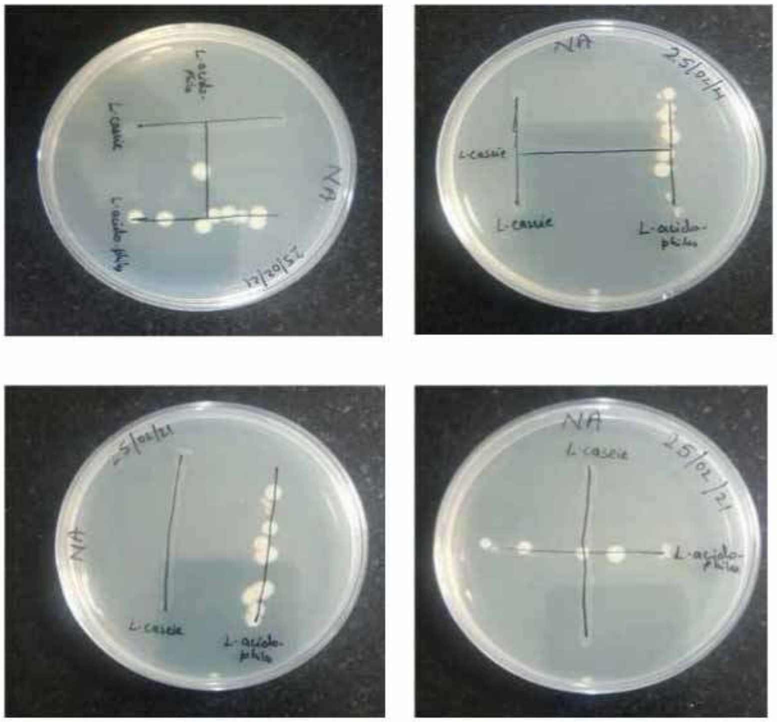


Fig. 1 Cross streaking of the selected Bacterial Strains

Enumeration of Bacteria for Culturing the Legume Milk - The revived lyophilized bacterial strains were serially diluted ten-folds and by using the pour plating method and incubation at 37°C for 24 hours, the enumeration of the bacterial density was carried out. All enumeration of the selected probiotics was done in 0.5 per cent peptone water modified MRS medium (Fig No. 2). The results of the cross streaking allowed for the preparation of co-cultured probiotic extracts. The probiotic strains and their density (in CFU/mL) used is mentioned in the Table No. 3.

Table 3
Bacterial colonies Used for Culturing the Legume Milk

S.No.	Probiotic Strain	CFU/mL
1	Lactobacillus casei NDRI strain RTS	33×10^6
2	Lactobacillus acidophilus NDRI HI 39	14×10^6
3	Bifidobacterium bifidum NCDO 2715	20×10^6
4	Lactobacillus casei NDRI strain RTS + Bifidobacterium bifidum NCDO 2715	16×10^5 + 13×10^5
5	Lactobacillus casei NDRI strain RTS + Lactobacillus acidophilus NDRI HI 39	20×10^5 + 14×10^5

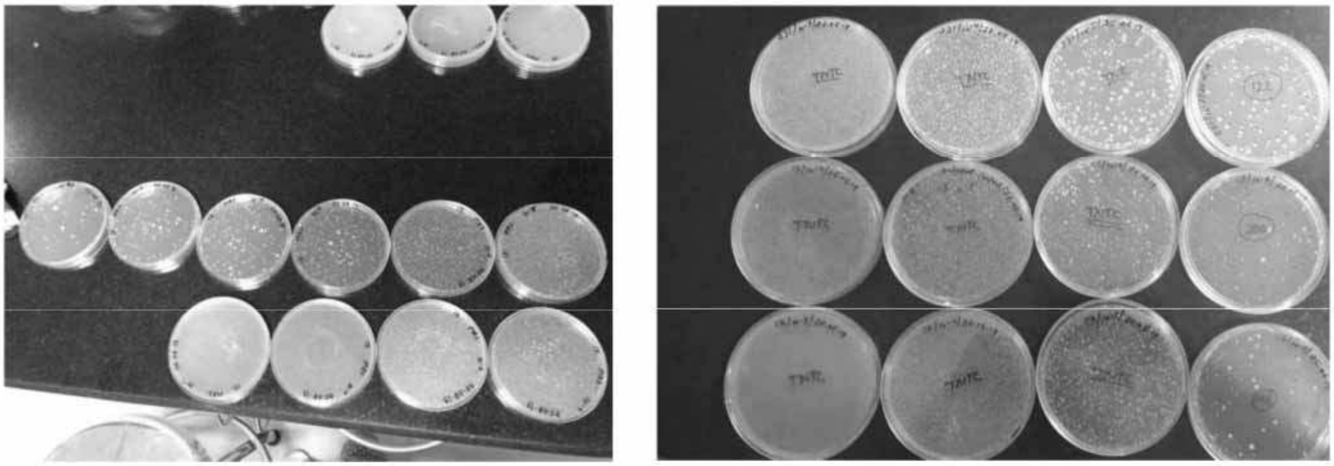


Fig. 2 Enumeration of the Probiotic Bacteria

The inoculum used was 1 mL for both mono culture and co-cultures. The trials were made on 10 mL of ChP and Cowpea milks. Fermentation was done for 24, 48 and 72 hours to see the increase or decrease in the bacterial densities and also, to set the time period needed for fermentation to attain the final product with bacterial density not less than 10^6 (Fig No. 3).

Fig. 3 Fermentation of Chickpea milks to enumerate the selected Bacteria

(iii) Development of probiotic variations of Chickpea milk with mono and co-cultures of the selected probiotic bacteria

The final results of the bacterial density are represented in the Table No. 4. With the passage of time from 24 hours to 48 and progressing towards 72 hours, the bacterial densities increased exponentially, producing a fermented extract (Fig No. 4) with high bacterial loads. The average density of probiotic bacteria after 24 hours ranges between10^3 to 10^4 CFU/mL, whereas after the time duration of 72 hours, the average count of the probiotic bacteria increased to the range of 10^6 to 10^8 CFU/mL.

Table 4
Total Count of Bacterial Densities in the Chickpea milk

S.No.	Probiotic Strain	Bacterial Densities of Chickpea milk after the incubation period of-		
		24 hours (CFU/mL)	48 hours (CFU/mL)	72 hours (CFU/mL)
1	Lactobacillus casei NDRI strain RTS	31×10^3	53×10^5	51×10^6
2	Lactobacillus acidophilus NDRI HI 39	29×10^3	57×10^5	43×10^6
3	Bifidobacterium bifidum NCDO 2715	15×10^4	28×10^6	31×10^7
4	Lactobacillus casei NDRI strain RTS + Bifidobacterium bifidum NCDO 2715	64×10^3	58×10^5	72×10^7
5	Lactobacillus casei NDRI strain RTS + Lactobacillus acidophilus NDRI HI 39	32×10^4	43×10^6	123×10^8

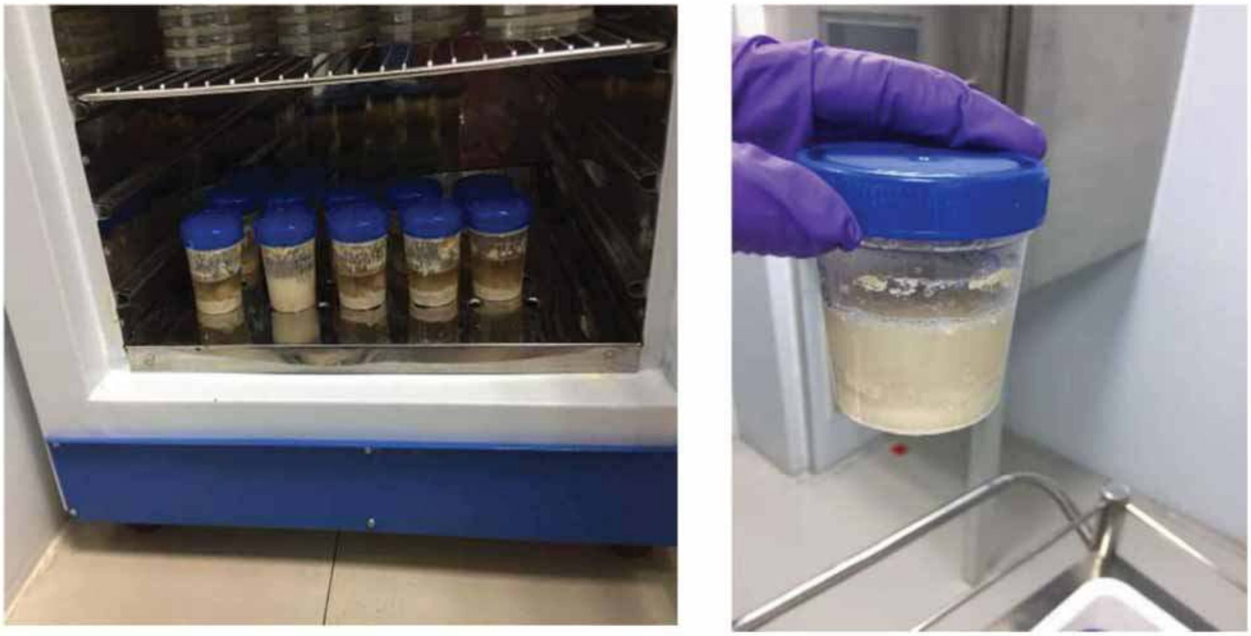


Fig. 4 Legume Milks after Fermentation

(iv) Assessment of organoleptic properties and statistical analysis of the Chickpea probiotic extracts

Five variations of the fermented extracts were developed with the mono and co-cultures of the probiotic bacteria and all these were evaluated for their organoleptic parameters like appearance, colour, texture, taste, aftertaste and overall acceptability by the selected panel members. The developed extracts were compared against the fermented beverage readily available by the brand name, YAKULT (control). **5 Point rating scale** was used to analyse the sensorial parameters of the fermented extracts and the results obtained are presented in the Table No. 5. The parameter of appearance was found to be significantly different at 0.01 level of significance (p=0.004). The maximum score was obtained by the control i.e., 4.87, followed by 4.73 which was obtained by the milk fermented with Lactobacillus casei NDRI strain RTS. The minimum score (3.80) was obtained by the extract prepared after the fermentation of cowpea milk with Bifidobacterium bifidum NCDO 2715.

The parameters of texture and taste were found to be significantly different at 0.05 level of significance where the p-values were 0.018 for texture and 0.033 for taste. The fermentation carried out by using Lactobacillus casei NDRI strain RTS was found to be the most acceptable as the scores for both texture and taste was 4.73.

Table 5
Comparison of Organoleptic Properties of Fermented ChP Milks developed from selected Probiotic Strains

	Control	**L. acidophilus**	**L. casei**	**B. bifidum**	**L. casei & B. bifidum**	**L. acidophilus & L. casei**	**F Value**	**p value**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**		
Appearance	4.87^{a} ±0.35	4.00^{b} ±0.38	**4.73^{a} ±0.46**	3.80^{b} ±0.41	3.47^{bc} ±0.64	4.13^{c} ±0.35	5.46**	0.004
Colour	4.93^{a} ±0.26	4.20^{b} ±0.41	**4.87^{a} ±0.35**	4.00^{bc} ±0.53	3.73^{c} ±0.46	4.27^{b} ±0.46	19.20***	0.000
Texture	4.80^{a} ±0.41	3.93^{b} ±0.70	**4.73^{a} ±0.59**	3.33^{b} ±0.49	3.60^{b} ±0.83	3.73^{b} ±0.59	3.43*	0.018
Taste	4.73^{a} ±0.59	3.80^{b} ±0.67	**4.73^{a} ±0.59**	3.93^{b} ±0.46	4.07^{b} ±0.46	4.33^{ab} ±0.72	6.92*	0.033
After Taste	4.80^{a} ±0.41	4.07^{b} ±0.45	**4.67^{a} ±0.61**	3.40^{c} ±0.51	4.00^{b} ±0.65	3.80^{bc} ±0.67	13.22***	0.000
OA	4.80^{a} ±0.41	4.07^{bc} ±0.46	**4.73^{a} ±0.45**	3.53^{c} ±0.64	3.87^{bc} ±0.64	4.13^{b} ±0.46	14.34***	0.000

	Control	L. acidophilus	L. casei	B. bifidum	L. casei & B. bifidum	L. acidophilus & L. casei	F Value	p value
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**		
Appearance	4.87^{a} ±0.35	4.00^{b} ±0.38	**4.73^{a} ±0.46**	3.80^{b} ±0.41	3.47^{bc} ±0.64	4.13^{c} ±0.35	5.46**	0.004
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- **Mean values represent the average of 15 determinants**
- **a, b, c, d - The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level**
- **Level of significance in increasing order – (*$p<0.05$, **$p<0.01$, ***$p<0.001$)**

Hedonic Rating Scale was employed to ascertain the overall preference of the panel members (n=15) for fermented ChP milk with reference to the fermented drink (YAKULT) available in the market. The results obtained are presented in Fig No. 5 and it can be concluded that the fermented ChP extract developed by using Lactobacillus casei NDRI strain RTS was found to be the most acceptable by all the panel members. Majority (66.66 per cent) of the panel members liked the extract very much and 26.66 per cent liked it moderately. The comparisons were made by using Yakult as a control. The extract prepared by using Bifidobacterium bifidum NCDO 2715 was liked slightly by only 7 panellists, 6 were neutral about the drink and 2 disliked the probiotic extract slightly.

The results obtained on hedonic scale and 5-point scale were found to be similar with regards to the preference of the fermented extracts.

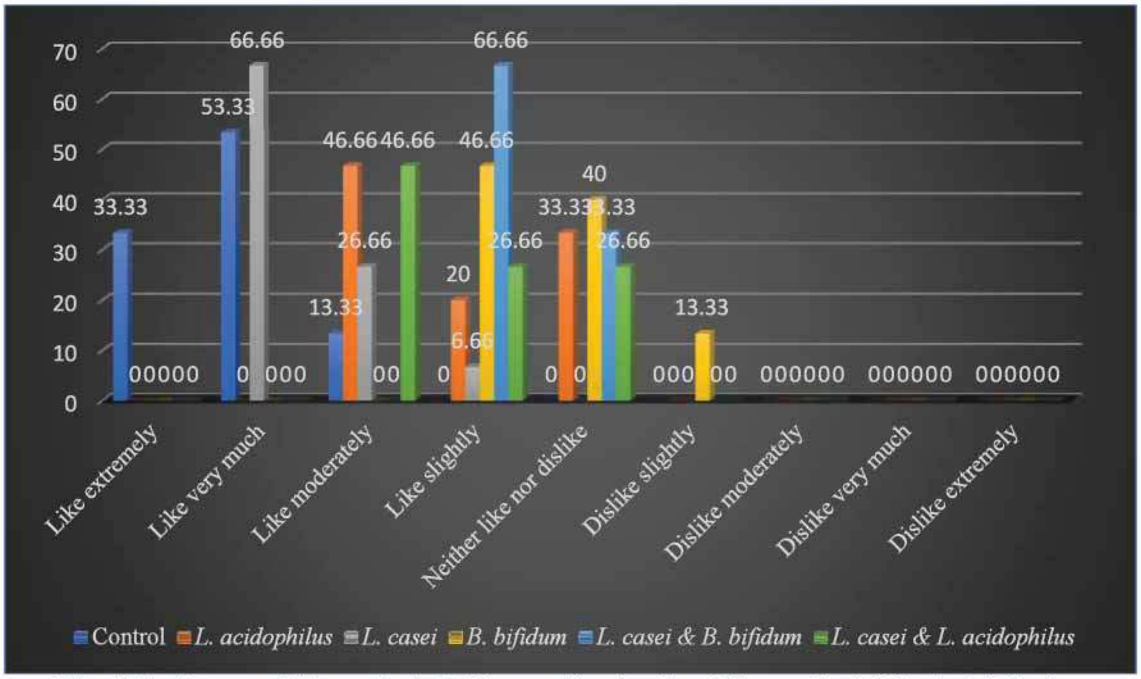

Fig. 5 Preference of Fermented Chickpea milks developed from selected Bacterial Strains

The resultant fermented beverage was well appreciated by the panellists, which could have been attributable to the predominant microorganisms throughout fermentation, lactic acid bacteria. During fermentation, lactic acid bacteria introduce a variety of antimicrobials and inhibitory compounds, which adds to flavour enrichment [26]. Lactobacillus and Bifidobacterium constitute the maximum number of lactic-acid producing bacteria which are commonly associated with the food fermentation processes [27].

Lopes et al. [4] attempted the development of a novel pulse-based beverage from ChP and lupin with various alluring characteristics (e.g., protein content, rheological behavior, color and appearance) and recommended it in the league of current commercial non-dairy beverages.

Zhang et al. [28] extracted milk from untoasted ChP and yam and later, fermented it with Lactobacillus casei. Besides being organoleptically acceptable, lactic acid bacteria fermentation improved the physico-chemical properties of ChP milk and enhanced its antioxidant activity. Ricci et al. [29] fermented cherry juice with Lactobacillus casei and as a result of the fermentation, there was a noticeable decrease in the sucrose concentration and increase in the total volatile compounds, especially propyl acetate. The beverage displayed fruity notes conferring due to lactic acid fermentation. Organoleptically, the probiotic beverage was found to be extremely acceptable.

Although the secondary data available on the legumes selected for the current study is meagre, but the probiotics used in the current study are the commonly employed probiotics and the acceptability by the consumers was also reported to be very high as mentioned in the studies conducted by various researchers on different substrates. This proves the novelty of the research giving it a new edge, and also proving for a source of probiotic to people with celiac diseases, lactose or gluten intolerance and an effective drink to improve the gut and overall health.

(c) Assessment of the Organoleptic Properties of Synbiotic Drinks prepared from Chickpea Probiotic Extract enriched with Prebiotic- CRP

The ChP extracts, obtained after soaking and blanching, were inoculated with mono and co-cultures of probiotic bacteria and five variations were developed. The variations were subjected to organoleptic evaluation by the semi-trained panel members (n=15). The most acceptable probiotic drinks were prepared by fermenting ChP extract with Lactobacillus casei NDRI strain RTS. The probiotic drinks were homogenized and added with food stabilizer i.e., pectin in 0.05 and 0.03 per cent concentration in ChP probiotic drinks respectively. After this, the ChP probiotic drink was sweetened with 7.5 per cent of powdered sugar was added. 0.03 per cent pectin was also added s a foo stabilizer. The obtained drinks were then blended thoroughly (Fig No. 6).

Fig. 6 Homogenized Probiotic Extracts developed from Chickpea Milks

The probiotic ChP extract was enriched with prebiotics in the concentrations of 3 per cent, 6 per cent and 9 per cent for developing three variations. The variations were developed by adding the prebiotic solely and in combinations. The final synbiotic drinks were subjected to organoleptic evaluation on a 5-point rating scale. The synbiotic drinks were organoleptically evaluated on a 5-point rating scale by 15 semi-trained panel members. The obtained results were presented as their means and the parameters were statistically evaluated by using ANOVA. The organoleptic parameters tested were appearance, colour, taste, texture, after taste and overall acceptability. The effect of different variations on the mean scores of organoleptic characteristics of ChP (CRP) synbiotic drink are presented in Fig No. 7 and the results depict that the 3 per cent CRP was found to be the most likeable drink.

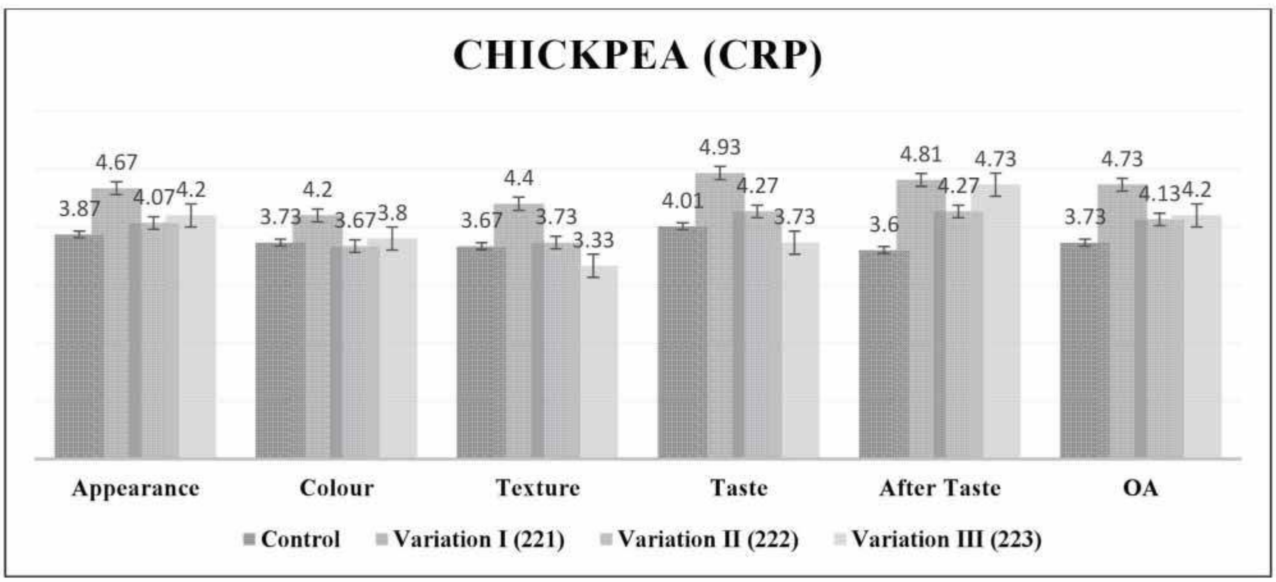

Fig. 7 Mean scores of Organoleptic parameters of different variations of ChP(CRP) Synbiotic drink

(d) Consumer Acceptability and Market Potential of the Developed Synbiotic Drinks

Consumer is broadly any individual that uses goods and services produced within the market. The concept of a consumer arises in diverse framework, so that the usage and significance of the term may differ [30].

In this section, an attempt was made to determine the acceptability of different variations of the synbiotic drinks by the consumer and its market potential. A random sample of 100 consumers were chosen for the study. Out of all the 100 subjects, 27 per cent belonged to the age group of 10-19 years, 39 per cent were in the age group of 20-39 years, 26 per cent in the age slot of 40-59 years and 8 per cent were above the age of 60 years. Out of all these subjects, 58 per cent were females and 42 per cent were males.

(i) **Organoleptic Evaluation of the Prepared Synbiotic Drinks -** The organoleptic evaluation of all the developed variations of synbiotic drinks prepared from the legume extracts was carried out by the consumer panel. The tool used for the organoleptic evaluation was Hedonic Rating Scale and only four parameters i.e., appearance, colour, flavor and mouthfeel were evaluated by the consumer panelists. The organoleptic scores of the synbiotic drinks prepared from ChP probiotic extract containing different proportions of prebiotic CRP varied significantly (Table No. 6). The appearance of the drinks, varied significantly ranging from 7.15 (111) to 8.41 (221). The choice of colour depicted significant difference ($p<0.001$). It was observed that 221 (8.49) was found to be highly acceptable followed by 222 (7.66), 223 (7.32) and 111 (7.15). Significant difference in the flavour of the different variations of synbiotic drinks was observed. 221 (8.51) had the highest score for flavour, followed by 222 (7.68) and 223 (7.38). Least was scored by 111 (7.17). The scores of mouthfeel for different variations were observed in the range of 7.13 to 8.62, which were found to be significantly different from each other ($p<0.05$). Maximum score was obtained by 221 i.e., 8.62. So, finally it can be summarized that 221 containing 3 per cent of CRP was the most liked synbiotic drink by the consumers.

Table 6
Consumer acceptability based on the organoleptic properties of Synbiotic drinks developed from ChP(CRP)

Variations / Parameter	111 Control Mean ±SD	221 3% Mean ±SD	222 6% Mean ±SD	223 9% Mean ±SD	F value	p value
Appearance	7.15^{c} ±1.07	**8.41^{a} ±0.53**	7.62^{b} ±0.91	7.35^{bc} ±1.07	3.90*	0.024
Colour	7.15^{c} ±1.40	**8.49^{a} ±0.50**	7.66^{b} ±1.01	7.32^{bc} ±1.18	6.43***	0.000
Flavour	7.17^{c} ±1.42	**8.51^{a} ±0.65**	7.68^{b} ±1.07	7.38^{bc} ±1.28	2.65*	0.049
Mouthfeel	7.13^{c} ±1.36	**8.62^{a} ±0.51**	7.64^{b} ±1.18	7.39^{bc} ±1.15	3.49*	0.018

- **Mean values represent the average of 100 determinants**
- **a, b, c – The non-identical letters in any 2 columns within the rows denote a significant difference at a minimum of 5% level**
- **Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)**

(ii) Market Potential of the Prepared Synbiotic Drinks - A consumer panel consisting of hundred subjects belonging to different age group of 10-19 years, 20-39 years, 40-59 years and greater than 60 years were subjected to evaluate the future of the synbiotic drinks if launched commercially into the market. The market potential was studied in terms of expected cost, frequency of purchase, novelty of the idea and need of the product, recommendation of the synbiotic drinks to others and the overall experience of the consumers related to the drink.

(iii) Consumer preference related to the cost of the developed synbiotic drinks - Results showed a significant difference ($p<0.001$) in the consumer preference with regard to the cost of the developed synbiotic drinks. Table No 7 revealed that the consumers were willing to buy the synbiotic drinks, if commercially available in the market. Preferred price range was between Rs.10 (by 52% consumers) and Rs.15 (by 31%). Only 9% of them prefereed to buy the drinks at Rs.20 also. However, some customers (8%) were willing to buy it even at a price of Rs.25/Unit.

Table 7
Consumer preference for the cost of synbiotic drinks

COST (in Rs.) / AGE GROUP	Rs.10	Rs.15	Rs.20	Rs.25	Chi Square (χ^2)	p value
10-19 years (n=27)	12(45)	10(37)	3(11)	2(7)	6.63	0.000
20-39 years (n=39)	21(54)	12(31)	3(7)	3(8)		
40-59 years (n=26)	15(58)	7(27)	2(7)	2(8)		
Above 60 years (n=8)	4(15)	2(25)	1(12)	1(13)		

Note: Figures in parenthesis indicate percentages

(iv) Consumer preference on recommendation of the Developed Synbiotic drinks to others - Results depicted in Table No. 8 revealed that 89% of the consumers preferred to recommend the product to others, thus depicting their interest and understanding about the need of the product. The results were found to be significantly different ($p<0.001$). The age-wise recommendation of the drinks was 93% (10-19 years), 87% (20-39 years), 88% (40-59 years) and 87% (above 60 years).

Table 8

Consumers' preference related to the recommendation of the Synbiotic drinks to Others

RECOMMENDATION / AGE GROUP	YES	NO	Chi Square (χ^2)	p value
10-19 years (n=27)	25(93)	2(7)	19.51	0.000
20-39 years (n=39)	34(87)	5(13)		
40-59 years (n=26)	23(88)	3(12)		
Above 60 years (n=8)	7(87)	1(13)		

Note: Figures in parenthesis indicate percentages

(v) Consumer preference based on the frequency to purchase the Developed Synbiotic Drinks - The results were found to be significantly different ($p<0.001$). Table No. 9 revealed that 69% of the consumers would prefer to purchase the developed synbiotic drinks on daily basis, whereas 19% and 12% of consumers may buy the drinks on weekly and monthly basis respectively. Out of the 69% consumers, who were willing to purchase the drink daily, were in the age-group of 10-19 years.

Table 9

Consumers Preference based on the Frequency to Purchase the Synbiotic drinks

FREQUENCY / AGE GROUP	DAILY	WEEKLY	MONTHLY	Chi Square (χ^2)	p value
10-19 years (n=27)	21(78)	4(15)	2(7)	8.33	0.000
20-39 years (n=39)	29(74)	7(18)	3(8)		
40-59 years (n=26)	15(58)	7(27)	4(15)		
Above 60 years (n=8)	4(50)	1(12)	3(38)		

Note: Figures in parenthesis indicate percentages

(vi) Consumer Preference on the Novelty of the Developed Synbiotic Drinks - Consumer views were studied about the idea of the new synbiotic drinks. The results were found to be significantly different ($p<0.001$). It was found that more than 72% subjects from all the age groups considered the drinks as a good idea and 22% found this novel synbiotic drink as an interesting product (Table No.10).

Table 10

Consumers opinion on the Novelty of the Synbiotic drinks

IDEATION / AGE GROUP	GREAT IDEA	SOUNDS INTERESTING	NEUTRAL	Chi Square (χ^2)	p value
10-19 years (n=27)	25(92)	1(4)	1(4)	11.57	0.000
20-39 years (n=39)	27(69)	10(26)	2(5)		
40-59 years (n=26)	17(65)	7(27)	2(8)		
Above 60 years (n=8)	3(37)	4(50)	1(13)		

Note: Figures in parenthesis indicate percentages

(vii) Consumers' overall experience related to the developed synbiotic drinks - Smiley scale was used to assess the overall experience of the consumers related to the developed synbiotic drinks and the results are shown in Table No. 11.

Table 11
Consumers' Overall Experience of synbiotic drinks

OVERALL EXPERIENCE / AGE GROUP	 (1)	 (2)	 (3)	 (4)	 (5)	Chi Square (χ^2)	p value
10-19 years (n=27)	0(0)	0(0)	1(3.73)	14(51.85)	12(44.44)	11.04	0.000
20-39 years (n=39)	0(0)	0(0)	1(2.56)	11(28.20)	27(69.23)		
40-59 years (n=26)	0(0)	0(0)	3(11.53)	8(30.76)	15(57.69)		
Above 60 years (n=8)	0(0)	0(0)	2(25.00)	3(37.50)	3(37.50)		

Note: Figures in parenthesis indicate percentages

In the present study, it was observed that 57% of the consumers liked it extremely (5), amongst this, most of the consumers were in the age group of 20-39 years. 31% of the consumers rated it as very likeable (Table No. 11). In this category, majority of the consumers belonged to 10-19 years of age. Only a very small proportion of consumers i.e., 7% remained neutral i.e., they were neither interested in buying nor drinking the synbiotic drinks, irrespective of its health benefits. The results were found to be significantly different ($p<0.001$).

Prebiotics are either natural or synthetic non-digestible (non) carbohydrate substances that boost the proliferation of gut microbes. Undigested fructo-oligosaccharides in the large intestine are utilized by the beneficial microorganisms for the synthesis of short-chain fatty acids for their own growth. Although various food products are now recognized as having prebiotic properties, such as almonds, artichoke, barley, chia seeds, chicory, banana, dandelion greens, flaxseeds, garlic, and oats. Considering the benefits of these prebiotics in mineral absorption, metabolite production, gut microbiota modulation, and in various diseases like diabetes, allergy, metabolic disorders and necrotising enterocolitis, increasing attention has been focused on their applications in both food and pharmaceutical industries, although some of these food products are actually used as food supplements [31]. When digestive enzymes hydrolyze prebiotic dietary fibres, the fermentation products in the colon seem to be mostly short-chain fatty acids (SCFAs) [31]. The end products of intestinal microbial fermentation of dietary fibers and resistant starches are the SCFAs, such as acetate, butyrate and propionate [33,34].

Non-digestible polysaccharides designated as resistant starches are being used as dietary fibre in the gastrointestinal tract and are fermented in an anaerobic environment by colonic bacteria to produce SCFAs [35,36]. The involvement of SCFAs in physiological equilibrium regulation has lately been widely investigated. SCFAs could be used as dietary interventions to reduce the risk of developing obesity, metabolic disorders, and ailments such as Type II diabetes and hypertension, due to its significance in energy homeostasis [37].

A study conducted by Decsi [38] stated that daily consumption of chicory root fibre in children aged three to six years, can keep the level of bifidobacteria higher and more stable, reducing the antibiotic-induced disturbances of the microbiota composition. In general, the children undergoing antibiotic treatment showed a reduction of microbiota, including bifidobacteria. However, the children also receiving the prebiotic supplementation demonstrated a significantly higher presence of bifidobacteria versus the control.

Azpiroz et al. [39] determined the effect of a prebiotic chicory-derived inulin-type fructan on the tolerance of intestinal gas. The results demonstrated that inulin decreased gas retention during the gas challenge test. The conclusions of this study were that a daily dose of inulin can promote bifidobacteria growth and may improve the overall gut function.

Kumar et al. [40] prepared a yoghurt ice cream by using buttermilk and chicory root extract (2-6%). The optimum conditions generated from the analysis were 0.25% stabilizer, 0.25% emulsifier, 26.43% buttermilk and 4% dried chicory root extract. The predicted response in terms of colour, flavour, texture, sweetness, hardness, melting rate, overrun and Overall Acceptance (OA) score were 7.22, 7.15, 7.42, 6.69, 40.16, 27.92, 40.09 and 7.200, respectively. The desirability of the optimum conditions was 0.83. The final product was found to be acceptable on all the organoleptic parameters.

Marteau et al. [41] investigated the impact of the daily consumption of 15 gm chicory native inulin on fecal levels of bifidobacteria, stool parameters and quality of life of constipated elderly in a randomized, double-blind, controlled versus placebo clinical trial. The impact of supplementation on constipation was assessed with questionnaires on bowel motor function parameters, visual analogue scales on bowel symptoms and quality of life. Fecal bacterial counts were determined at the beginning and at the end of the supplementation. The results revealed that inulin supplementation led to a significant increase in total fecal bacteria and bifidobacteria concentrations after 28 days of consumption. The volunteers in the inulin group reported increased satisfaction about digestion and reduced defecation difficulties during the supplementation. Slight gastrointestinal symptoms (flatulence) were reported but did not lead to discontinuation. This study concluded that daily supplementation with 15 gm of inulin improves the quality of life in an elderly population suffering from constipation.

(e) **Analysis of physico-chemical, nutritional, microbial, antioxidant and probiotic properties of the highly acceptable drink -** Analysis of the physico-chemical, nutritional, microbial, antioxidant and probiotic properties of the highly acceptable drinks developed from was carried out. This phase concluded that the pH and viscosity of the synbiotic drink was 4.37 and 5.39 mPa.S@20°C respectively. The percent acidity was reported to be 0.65% and the peroxide value was 6.27. The **nutritional qualities** of the highly acceptable drink reported that the moisture content of the drinks was 79.52 per cent. The crude fat content was found to be 0.98. The drink contained 3.29 g/100mL of fiber and 3.87 g/100mL of protein. The ash content was reported to be 2.78 per cent. As far as the carbohydrate content was concerned it was 16.06 g/100mL and the total calorie content was 84.54KCal/100mL. The **microbial studies** of the highly acceptable synbiotic drink revealed that the Total Plate Count, in $\log^{10}$ CFU/mL, was found to be 8.09. The Yeast and Mold Count was found to be <1 CFU/mL and the Total Coliform Count was reported to be negligible. **Total Antioxidant Capacity (TAC)** of the developed drink was determined by using DPPH assay and was recorded to be 42.32 mg TE/100g. The **probiotic properties** of the drink was assessed by using in vitro acid and bile acid simulation. The two strains of LAB i.e., Lactobacillus casei and Lactobacillus acidophilus, were tested for survival percentages under the simulated acidic and bile conditions. Both the strains have shown survival percentages of 95.81 and 98.89 respectively, to the simulated acidic conditions. Under the simulated bile conditions, the survival percentages of Lactobacillus casei and Lactobacillus acidophilus were reported as 84.88 and 89.52 per cent respectively.

(f) **Development of food label, aseptic bottling, shelf-life and cost analysis of the packaged drinks**The **labels** were designed by using CorelDRAW keeping in mind the specifications given by the Food Safety and Standards (Packaging and Labelling) Regulations, 2011. The drink developed from the legume was **packaged** in PET bottles and shelf-life analysis was carried out for a period of 60 days at an interval of 10 days. The shelf-life studies were conducted for the organoleptic, physico-chemical and microbial parameters of the developed and packaged synbiotic drinks. The **organoleptic properties** of the prepared synbiotic drinks were tested by composite scoring test and the drink was found to be likeable on the parameters of colour, consistency, aroma, taste and flavour, mouthfeel, after taste, absence of defects and overall acceptability for a period of 40 days, when kept under refrigerated conditions. The **physico-chemical parameters** that were tested for 60 days were pH, viscosity, acidity and peroxide value. A general trend was recorded in all these parameters i.e., pH decreased with time; and percent acidity, viscosity and peroxide value increased with the storage time. The **microbial parameters** were also stipulated and Total Plate Count increased initially for a period of 30-40 days and then, decreased thereafter. The Yeast and Mold Count was recorded to be <1 CFU/mL and negligible Total Coliform Count was observed during the total storage period of 60 days in the developed synbiotic drink indicating the use of good hygienic practices during the development of the drinks. The **resultant cost** of Rs. 9/Bottle was calculated for the final synbiotic drink (Fig No. 8).

Fig. 8 Packaged Bottle of Synbiotic Drink prepared from Chickpea probioticextract enriched with Chicory Root

IV CONCLUSION

To conclude, Chickpea was selected for the current study for the development of legume-based milk. To obtain milks, the legume was processed by employing four processing methods to ascertain an organoleptically acceptable milk as compared to the legume milk (soy milk, SOFIT) available commercially. The processing method of soaking followed by blanching the legumes was reported to be the best acceptable technique for the development of legume milk. Even the by-products procured, after the extraction of milks, were utilized to prepare Hummus. Further, the best acceptable milks were subjected to fermentation by LAB i.e., Lactobacillus casei NDRI strain RTS, Lactobacillus acidophilus NDRI HI 39 and Bifidobacterium bifidum NCDO 2715, in mono and co-cultures. The ChP milk fermented by employing mono culture of Lactobacillus casei NDRI strain RTS was found to be most acceptable organoleptically on all the parameters. The extracts were enriched by employing CRP. Three variations were prepared by using 3, 6 and 9 per cent of prebiotics. The probiotic extract enriched with 3 per cent of CRP was found to be highly acceptable on all the organoleptic parameters. The quality analysis of the drink revealed good physico-chemical and nutritional properties, with low fat, moderate protein and carbohydrate contents. High ash content of the drink revealed that drink possess good mineral content as well, owing to the utility of CRP. The synbiotic drink was reported to have a microbial load of 10^8, as recommended by WHO, 2021. The drink was not only palatable, but also reported to have high antioxidant and probiotic properties. Thus, from the overall study, it was concluded that the ChP based synbiotic drink was highly acceptable and have huge market potential. The shelf-life of the drinks was reported to be the best, organoleptically, physico-chemically and microbiologically, till 40 days of storage at temperature ±4°C. The cost of the developed drink came out to be Rs.9 which is affordable by the people of all socio-economic background.

The developed drink fulfilled the criteria of pro- and prebiotic properties, had good overall acceptability and fits in the budget of people belonging to various socio-economic status, proving its beneficial effect on people of all age groups. So, this work strongly contributes to pave the way for the development of novel legume-based synbiotic drinks with several appealing features (e.g., pro- and prebiotic quality, taste and flavour, protein content, colour and appearance) that are highly competitive in the current commercial non-dairy beverages. Thus, the developed novel legume-based synbiotic drinks could be recommended for large scale production at Industrial level.

V RECOMMENDATIONS FOR THE POLICY MAKERS

As the incidence of under nutrition among children is still prevailing in our country, and one of the major causes of it, is poor gastro-intestinal health. So, the policy makers of the initiatives taken by the Indian Government like National Nutrition Policy, Mid-day Meal programme, *POSHAN Abhiyan etc.,* can be approached for the creation of awareness about the health benefits of synbiotic drink and can be suggested to introduce such drinks. Also, when the drink will be prepared at large scale, the cost of the drink will be reduced drastically.

The by-products of Chickpea can be employed in the making of ready-to-eat value added products or can also be dried and powdered. The powder can be used in various recipes like *chapatti*, pizza, dhokla, cookies, breads *etc.,* owing to its high nutritional value and functional properties. Furthermore, the by-products of Chickpea, produced post-extraction of legume milk, can be subjected to fermentation and can be used as a bio-fertilizer, being rich in micronutrients like nitrogen, potassium and phosphorus. These effective nutrients of the infilterate can prove to be a successful organic fertilizer, on the basis of prolonging the utilization chain of resources, making it profitable and environment friendly.

Currently, research in this area is occurring at a brisk pace, but the scope looks pretty wide. So, there is a need to develop novel and economical synbiotic foods, considering their potential health benefits, especially for people suffering from lactose intolerance cardio-vascular diseases and people following FAD lifestyles.

Patent: "Non-dairy Synbiotic Beverage and the Process" under **The Patents Act, 1970 (39 of 1970) & The Patents Rules, 2003** was filed via application number **201911051242** on 11 December, 2019. The patent was also published in the **Journal of Patents (U/S 11A)** on 18 June, 2021.

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