Full Length Research Article

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Research Journal of Agricultural Sciences

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

National Conference on "Emerging Technologies Focusing on Food Safety and Security"
Volume 15; (March 2024); pp 120–128

In vitro Probiotic Efficacy of Bacteria Isolated from *Cicer arietencium* Curd

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Abstract

The study focused on developing vegan curd from chickpeas and evaluating its probiotic efficacy. Microbial analysis showed a total viable count of 1.52×10^6 CFU/ml, with no presence of yeast, mold, or E. coli. Using a nine-point hedonic scale, organoleptic evaluation demonstrated significant acceptability based on sensory attributes like appearance, colour, taste, texture, flavour, and overall acceptability. Proximate composition analysis revealed energy content (191 Kcal), carbohydrates (12.11 g), protein (35.1 g), fat (1.24 g), fiber (5.33 g), calcium (49.04 mg), and vitamin C (195 mg). Additionally, physical properties such as pH, colour, viscosity, titratable acidity, total soluble solids, and textural properties were assessed, along with a 7-day storage study. The vegan curd exhibited potential as a probiotic, richness in vitamin C, cost-effectiveness, and microbial safety, making it a promising option for individuals with dietary restrictions or preferences seeking alternatives to cow milk.

***Key words*:** Vegan curd, Probiotic efficacy, Chickpea, *Cicer arietencium*, Storage study

Plant-based milk substitutes have become more popular around the world as a result of their numerous health benefits. Phenolic compounds, unsaturated fatty acids, antioxidant activity, and bioactive chemicals such as phytosterols and isoflavones make plant-based milk substitutes an excellent choice, despite the added sugar and lack of total protein content. Lactose intolerance or allergies to cow's milk necessitate the use of plant-based milk alternatives. Because plant-based milk substitutes are utilized as a key ingredient in many vegan food items such as plant-based yogurt, cheese, kefir, butter, ice cream, and so on, they are one of the food groups that are irreplaceable in the vegan food business. Chickpea (*Cicer arietinum* L.) is a semiarid crop that belongs to the genus Cicer, tribe Cicereae, family Fabaceae, and subfamily Papilionaceae. It is cultivated primarily in semiarid areas. Chickpea is chosen as the most important food legume plant in a sustainable agricultural system because of its low production cost, wider adaptation, ability to fix atmospheric nitrogen, and flexibility to fit in diverse crop cycles. Chickpeas are a nutrient-dense and healthful food, with high protein content, and carbs, particularly in key amino acids, as well as fiber, vitamins, and minerals. Raffinose-family oligosaccharides (RFO), resistant starches, and fibers are abundant in chickpeas [18] which helps to lower total cholesterol and LDL cholesterol.

Chickpeas are abundant in linoleic and oleic acids, which are unsaturated fatty acids. Plant-based milk substitutes have health benefits such as antioxidant activity on the immune system and a lower risk of cardiovascular disease [45]. Vegan milk is a plant-based drink that has the same flavor, texture, and properties as regular animal milk. Plant milk strives to contain the same number of nutrients as animal milk, although vegan milk has a lower nutrient density than dairy milk because they are made from processed extracts of the original plant. Plant-based milk alternatives have a huge growth potential in the health food market, and they need to be thoroughly investigated through advanced processing, technological interventions, and fortification techniques to develop a nutritionally complete beverage with high overall consumer acceptability.

The term 'probiotic' is taken from the Greek language and means 'for life. It has been defined in several ways in the past. Definitions such as 'substances produced by protozoa that stimulate the growth of another' or 'organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance' were used. Live bacteria in probiotic goods, such as bio-yogurt, boost the health of the host by exerting beneficial effects on the gastrointestinal system. The consumption of probiotic bacteria in the form of food products is an excellent technique to rebalance the intestinal microbiota [15]. There are two types of probiotic products: non-fermented and fermented. Probiotic yogurts are included in the second category. It's critical to produce probiotic yogurt with the best possible survivability, sensory, and economic features [11]. Many different types of probiotic yogurt are produced around the world, each with a different cultural makeup. However, throughout the fermentation process and refrigerated storage, probiotic viability is lost, which is a key challenge in

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the manufacture of probiotic yogurt [14] [27]. The goal is to make them healthier and more similar to cow's milk. It's worth noting that most plant-based beverages can't replace cow's milk in terms of nutritional value. The type of plant-based beverage chosen will be determined by the person's goals (nutritional or sensory) as well as their tastes and limits [20]. The objectives of the study are to standardize chickpea curd and to determine the physical, and microbial loads of the formulated product. To analyze the sensory characteristics, shelf life, and invitro probiotic efficacy of the developed product.

MATERIALS AND METHODS

Procurement of raw material

Chickpea – Chickpea was procured from the local supermarket, in Chennai, India.

Pre-processing of raw materials

The procured raw materials are pre-processed before preparing the product.

Chickpea - Chickpea is washed with water to remove any dirt and it is soaked overnight for 8 hours.

Extraction of chickpea milk

Chickpea milk was extracted from the chickpea seeds. The chickpea was cleaned manually to remove dust. The cleaned chickpea 100g was soaked in 300ml cleaned tap water for 10-12 hours. Water and chickpea are separated. The soaked chickpeas are added to the blender to make it into a smooth paste. The paste was mixed with 300ml cleaned tap water to the thickness of the milk and sieved through a muslin cloth (1mm) into a fitted container.

Formulation of curd

Preparation of vegan starter culture

100g of chickpea was soaked for 8 hours. The chickpea was strained and the filtered water was used as a starter culture for the formulation of curd.

Preparation of chickpea curd

Chickpea milk was inoculated as described by [32], Chickpea milk was pasteurized for 15 minutes at 600C and allowed to cool. 2 ml of Starter culture was dissolved in lukewarm milk (37 degrees C) in a cup and stirred well. The milk was incubated at 35 °c for 10-12 hours.

Microbial analysis of the curd

Total plate count

The total plate count (TPC) of the spread sample was estimated by the procedure laid down in IS 1479 (part: III), 1997 using nutrient agar (NA) media.

Yeast and mold

Potato dextrose agar (PDA) was used to enumerate yeast and mold count as per the procedure laid down in IS: 1479(PART: III),1977.

Total viable count

DeMan-rugosa (MRS) agar was used to grow lactic acid bacteria. A series of dilutions using peptone water were prepared by aseptically removing the diluent of the culture until the dilution factor determined in the preliminary test was achieved. Using the spread plate method, 0.1 ml of the sample was transferred onto the agar. All the inoculated agar plates were left to incubate with AnaeroGen Sachet at 37 °C for 72 hours. The colonies were counted using a colony counter and expressed as CFU/ml.

Organoleptic evaluation of the curd

Sensory analysis is the process of inspecting a product using the five senses (sight, smell, taste, touch, and hearing) for quality features such as appearance, flavour, scent, texture, and sound. [40] It is analyzed using a 9-point hedonic scale.

The nine-point hedonic scale is the most prevalent, with 1 indicating extreme dislike and 9 indicating extreme like. The hedonic scale posits that participants' preferences are on a spectrum and that their responses can be divided into two categories: like and dislike. The curd was prepared with chickpea milk and subjected to organoleptic evaluation. The curd was organoleptically evaluated by 15 untrained panelists by using a 9-point Hedonic Rating Scale" at SDNB Vaishnav College for Women. The sensory scores of Curds for each parameter were subjected to statistical analysis to calculate mean scores.

Physical properties of the curd

Colour

Colour is an important quality indicator for curd. Instrumental colour analysis using the Hunter Colour Flex Colourimeter provides an objective way to quantitatively measure and compare the colour of curd samples, by standardizing the elements necessary to view colour.

Three elements are necessary to see colour: a light source, an object, and an observer. Three-dimensional scales, such as CIE L*a*b*, have been developed to objectively quantify colour values. This scale defines colour as follows:

L* (lightness) axis: black to white (0 to 100)
a* (red-green) axis: positive values are red; negative values are green; 0 is neutral
b* (yellow-blue) axis: positive values are yellow; negative values are blue; 0 is neutral

Viscosity

Viscosity is the measure of the resistance of a fluid to deform under shear stress. It is commonly perceived as flow behavior or resistance to pouring.

Titratable acidity

Around 10g of the sample was weighed and 30 ml of lukewarm water was added. Add one ml of phenolphthalein indicator solution. Shake well and titrate against standard sodium hydroxide solution. Stir vigorously throughout. Keep in another dish or basin about 10 g of the material diluted with 30 ml of lukewarm water as a blank for comparison of colour. The persistence of a slight pinkish tinge for 30 seconds indicates the endpoint The titration shall be preferably made in north light or under illumination from a daylight lamp. (IS 1166 – 1973)

Proximate composition of the curd

The proximate composition such as carbohydrates (AOAC 2000), protein [Kjeldahl method (AOAC 2002)], fat [Soxhlet method (AOAC 2000)], moisture (AOAC 2000), ash content (AOAC 2000), and calcium (AOAC 2000) of the chickpea curd was evaluated.

In vitro probiotic efficacy of the isolate

Isolation of lactic acid bacteria from curd

For isolating lactic acid bacteria curd sample was suspended, appropriately diluted in sterile normal saline, spread plated on de Mann Rogosa Sharpe (MRS) agar, and incubated

at 37°C for 2 days. Only two isolates were isolated because of the covid pandemic. The two isolated colonies were transferred to MRS broth and purified by streaking twice on an MRS agar plate.

Morphological identification

The two isolated organisms were subjected to the following morphological identification:

Spore staining

Gram-positive and catalase-negative isolates were grown on MRS agar at +37°C for 24 h under anaerobic conditions. The spore-staining procedure was applied. After the spore-staining technique, the endospore formulation was examined under light microscopy using oil immersion objectives. The isolates that did not form endospores were selected for further analysis.

Biochemical identification of the isolate

The two isolated organisms were subjected to the following biochemical tests:

KOH test

The KOH test was used to determine the gram reaction of LAB isolates. LAB cultures were grown on MRS agar at 37°C for 24 h under anaerobic conditions. The isolates, that did not give a viscid product, were selected since lactic acid bacteria (LAB) are known as Gram-positive cells.

Catalase test

Overnight cultures of isolates were grown on MRS agar at +37°C for 24 h under anaerobic conditions. The catalase test was conducted by dripping two drops of hydrogen peroxide (3%) on 24 hold cultures on a glass slide. The catalase test showed a positive reaction characterized by the formation of oxygen bubbles that indicate the production of catalase enzyme by the test bacterium. Therefore, the isolates, that did not give gas bubbles, were selected for subsequent activities. [30]

In vitro probiotic efficacy of the isolates

Tolerance to low pH

The isolates were grown separately overnight in 5 ml MRS broth at +37°C under anaerobic conditions. A volume of 1 ml of log 7 CFU/ml of each overnight-grown culture was inoculated into 10 ml of MRS broth to give an initial log 6 CFU/ml inoculum level. The culture was then centrifuged at 5000 rpm for 10 min at +4°C. The inoculated plates were incubated at 37°C for 24 to 48 h under anaerobic conditions using an anaerobic jar. The grown LAB colonies were expressed as colony-forming units per milliliter (CFU/ml). A positive control consisting of regular MRS broth inoculated with the culture was used. The survival rate was calculated as the percentage of LAB colonies grown on MRS agar compared to the initial bacterial concentration.

Tolerance to bile salts

To estimate bile tolerance of acid-tolerant LAB the isolates were separately grown overnight in MRS broth at 37°C under anaerobic conditions. The survival rate was calculated as the percentage of LAB colonies grown on MRS agar compared to the initial bacterial concentration.

Bacterial adhesion to stainless steel plates

Adherence assay of acid-bile-tolerant, antagonistic, and antibiotic-sensitive lactic acid bacterial isolates was determined on stainless steel plates.

Gas production from glucose

To determine LAB isolates' homofermentative and heterofermentative characteristics, CO_2 production from glucose was determined in modified MRS broth containing inverted Durham tubes with 1% glucose. The presence of gas in Durham tubes during 5 days of observation indicates CO_2 production from glucose [30].

Storage study of the curd

Storage studies were carried out for the prepared sample and quality parameters were evaluated during storage. The quality parameters of the sample produced were taken after preparation and the measurement was considered as the quality parameter on the 0th day. Later, the sample was filled in glass containers respectively. Containers were sealed tightly. The packed samples in a glass container were stored at a refrigerated temperature of 4 degrees Celsius. The quality parameters data were taken at regular intervals of the 0th day, 1st day, 3rd day, and 7th day.

Microbial attributes during storage of the curd

Microbial analysis during the storage period has been showed in (Table 1).

Table 1 Microbial analysis during the storage period

Shelf-life attributes	Reference
Yeast and oould	Hitching *et al.* (1995)
E. coli	Bellio *et al.* (2018)

Sensory evaluation during storage: Sensory evaluation was done on the 0th day, 1st day, 3rd day, and 7th day of the curd sample.

Statistical analysis of data

Planning, designing, collecting data, analyzing, making relevant interpretations, and publishing research findings are all statistical processes involved in conducting a study. Colour, taste, appearance, flavor, texture, and general acceptability were used to calculate the average and standard deviation. SPSS software was used to calculate the mean, standard deviation, and ANOVA. The statistical process of analysis of variance (ANOVA) is used to compare the means of several samples. The goal is to see if there are any significant differences in class means, which is done by the analysis of variances.

Cost calculation of the curd

The cost for each product is separately calculated. The food cost, the labour cost, the overhead cost, and the hidden cost are included under this analysis category. The cost analysis for the value-added curd was also done to check its affordability to the common people. The cost analysis is estimated through the following formula:

$$Cost\ of\ Production = \frac{COST\ A + COST\ B}{Q}$$

Were,
Cost A = Cost of the raw materials
Cost B = Cost of the processing.
Q = Quantity of the chickpea

RESULTS AND DISCUSSION

Microbial analysis of the curd

Microbial analysis of any food product, new or existing, aids in recognizing the quantitative and qualitative existence of microorganisms in the given food product. The formulated curd

was subjected to microbial analysis. The total microbial count of the curd control and sample was formulated in the given (Table 2). The probiotic viability for the curd sample was 1.52×10^6 CFU/ml, as lactic acid bacteria. When the viability count is compared to coconut milk yogurt it is 1×10^6 CFU/ml. the chickpea curd has better growth of probiotic bacteria. Hence, the growth of probiotic bacteria is dependent on the presence of sugar in the food product, which is available for fermentation and sustainability of probiotics. The microbiological quality was determined by enumerating its TPC. The total plate count of the sample was 1.850×10^4 CFU/ml. The microbial value of plain dairy yogurt was 6.81 $\times10^6$ CFU/ml. When compared with the above value, the sample was lower than the plain yogurt. The microbiological requirements, according to FSSAI 2011, are not more than 50 CFU/ml for total plate count (TPC). Hence, it is within the safe limit which is safe for human consumption. Yeast is a class of fungi that requires a warm and moist environment and a food source to grow. Yeasts are eukaryotic organisms that are included in a group of organisms called fungi, which also include molds. The yeast and mould count of the sample and control received nil growth due to proper hygiene and sanitation [14]. *E. Coli* is a type of bacteria that lives in the intestines and is responsible for some cases of food poisoning. It is clear from the table, that there is no evidential growth of coliforms in both the control sample and as well the sample because of the highly proper hygienic conditions and proper sanitation during the manufacturing of yogurt. Hence, it is safe for consumption [19].

Table 2 Microbial analysis of the curd

Parameters	Control	Sample
Total viable count	1.75×10^5 CFU/ml	1.52×10^6 CFU/ml
Yeast and mould	Nil growth	Nil growth
E. coli count	Nil growth	Nil growth

Organoleptic evaluation of chickpea curd

The curd was prepared with chickpea milk and subjected to organoleptic evaluation. The curd was organoleptically evaluated by 15 untrained panelists by using a 9-point hedonic Rating Scale. The different attributes considered for organoleptic evaluation are appearance, colour, taste, texture, flavour, and overall acceptability. The (Table 3) presents a correlation of the mean scores allotted by the panel judges for each sensory attribute of the products. The chickpea curd had a maximum overall acceptability score than cow milk curd. Then the chickpea curd was subjected to further analysis. Appearance is the first feature detected by the human senses is appearance, which plays a critical role in the identification and final selection of food. Colour, shape, size, gloss, dullness, and transparency all contribute to the visual experience of food [40]. The highest score for appearance was recorded for sample 8.4±0.50 when compared to the control curd 7.4±0.63. This indicates that the appearance of curd made from chickpea was highly acceptable than control by the untrained panelist.

Table 3 Mean acceptability and Standard deviation scores of curd

Parameters	Control	Sample	P-Value
Appearance	7.4 ± 0.63	8.4 ± 0.50	0.004**
Colour	7.06 ± 0.70	8.66 ± 0.48	0.001**
Taste	7.33 ± 0.48	8.73 ± 0.45	0.001**
Texture	7.4 ± 0.73	8.53 ± 0.51	0.001**
Flavour	7.06 ± 0.96	8.73 ± 0.45	0.001**
Overall acceptability	7.2 ± 0.77	8.8 ± 0.41	0.001**

*Significant at 5 %; NS – Not significant; **Significant at 1%

Colour is one of the major attributes which stimulates virtually all food appetites. The colour of the Sample (8.66±0.48) curd was found to be the highest acceptable sample compared to the control group. The colour attribute of the control group was found to be 7.06±0.70. The data shows that Sample was liked very much by the panelists on a 9-point hedonic scale whereas the control group was moderately accepted curd by the panelists. Taste is a key aspect in the acceptance of a food product and is one of the most essential considerations of organoleptic evaluation [40]. The score recorded for the developed product is shown in Table. From the table, it is revealed that the average score for a taste for developed sample curd was 8.73±0.45. The taste attribute of the control group was found to be 7.33±0.48. The data shows that Sample was liked very much by the panelists on a 9-point hedonic scale whereas the control group was moderately accepted curd by the panelists.

The texture is a prerequisite for the acceptability of a variety of foods. The consistency, thickness, fragility, chewiness, and size and form of food particles are all factors [40]. The texture attribute of the Sample was found to be 8.53±0.51 which is the highest acceptable sample. The texture attribute of the control group was found to be 7.4±0.73. The data shows that Sample was liked very much by the panelists. The flavour is a sensory phenomenon that is used to describe odour, taste, and mouthfeel experiences. Flavour is one of the most important factors in making the food product acceptable or disliked [40]. The flavor attribute of the Sample was found to be 8.73±0.45 which was very much liked by all panelists and gained the highest acceptability. The flavor attribute of the control group was found to be 7.06±0.96. The interaction of food with the consumers was related to the food product's overall acceptability. The overall acceptability rate of the Sample was 8.8±0.41 which was found to be the most acceptable curd The overall acceptability of the control group curd was 7.2±0.77. From the above-mentioned data, the overall acceptability was found to be highly acceptable than the control group.

Physical properties of the curd

The physical properties such as pH, total soluble solids, titratable acidity, viscosity, colour, and textural properties of the curd sample. The physical properties of the control and sample curd are given below in (Table 4).

Table 4 Physical properties of curd

Parameters	Sample	Control
pH	3.2±0.16	4.6±0.4
Titratable acidity (ml)	1.033±0.02	0.44±0.01
Viscosity (cp)	12.33±0.24	24.67±0.40
Total soluble solids (%)	21.2±0.86	12.5±0.34

pH

pH is an important parameter in the analysis of the concentration of hydrogen ions present in food products. The pH obtained for the sample was 3.2±was 0.16 and the for control 4.6±0.4 respectively when compared with soy milk yogurt 4.81±0.1 and coconut milk yogurt 4.5±0.1. The pH of the sample is affected due to the rapid fermentation of the sample with the production of lactic acid bacteria [13].

Titratable acidity

Titratable acidity is the measure to analyse the total acid produced in food products. The titratable acidity obtained for the curd sample was 1.033±0.02 ml. The sample has higher acidity when compared with coconut milk yogurt and soy milk

yogurt (0.44±0.01 ml and 0.25±0.43 ml). Hence, due to the rapid fermentation and growth of lactic acid bacteria, the sample showed higher acidity.

Viscosity

The viscosity of the curd has been estimated through a viscometer and the results of which have been provided in the table. Viscosity measurements are carried out to examine the food structure, which is associated with the flow of the fluid. It can be affected by temperature, particle size distribution, nature of the particle surface, particle shape, and volume of the dispersed phase. The viscosity of the sample 12.33±0.24 cp is lower than the control 24.67±0.40 cp. The sample shows a decrease in viscosity, which is a thixotropic behavior. This could be explained by the occurrence of syneresis during the processing of yogurt.

Total soluble solids

The total soluble solid is the measure to analyze the quality present in the given. The solid content of the sample 21.2±0.86 % is higher compared to the control 12.5±0.34 %. A similar study done by Shrishti 2019, found that 14.06±0.30 % in coconut milk yogurt. Hence, the soluble solids were slightly higher in chickpea curd which states that the quality of the chickpea curd is better and safe for consumption.

Table 4A Textural properties of curd

Parameters	Sample	Control
Firmness	3082.14	141.14±23.90
Adhesiveness	1.470.09	-193.32±17.51
Cohesiveness	-155.0116.72	0.81±0.07

Textural properties of the curd

The textural characteristics of curd samples formulated from chickpea milk are presented in (Table 4A).

Firmness is the most commonly assessed parameter for yogurt texture analyses it was defined as the necessary force to attain a given deformation. This factor is a critical texture property for yogurt-like products. The sample showed firmness of 141.14±23.90 whereas cow milk yogurt has shown higher hardness (308±2.14) because incubation time can negatively affect the textural properties of yogurt.

Adhesiveness or stickiness is the required work for the prevailing attraction force between foodstuff surfaces and various substances coming into contact with them. Adhesiveness is the force required to separate the material that sticks to the teeth during eating. The sample showed adhesiveness of -193.32 ± 17.51 which is compared to the control values which is 1.47±0.09. A similar study done by [18] found mean values for the adhesiveness of soymilk yogurts varied between -5.89 - -8.59 [5].

Cohesiveness, which is defined as the extent to which a material can be deformed before its rupture, depends upon the strength of internal bonds. The sample showed 0.81 ± 0.07 which is higher compared to the control -155.01±16.72 because the protein matrix of chickpea had an essential role in cohesiveness.

Colour analysis of the curd

The results of the colour analysis revealed that chickpea curd has lightness coordinates L*of 80.48 ± 0.43 compared to control curd of 73.06 ± 0.50. hence, the higher the L* value the lighter the sample (Table 4B).

Redness a* the sample curd was -1.93 ± 0.08 compared to the control curd -7.94 ± 0.0.07. The negative values obtained for the parameter a* indicated a green colouration of the curd.

Yellowness b* the sample chickpea curd has 21.66 ± 0.143 compared to the control curd 9.41 ± 0.07. This is due to the colour of the yellowish-white colour of chickpeas.

The total colour difference ΔE is an indication of the colour difference between the sample 68.51 ± 0.45 and the control 20.32 ± 0.47.

Table 4B Colour analysis of the curd

Parameters	Sample	Control
L*	80.48 ± 0.43	73.06 ± 0.50
A*	-1.93 ± 0.08	-7.94 ± 0.0.07
B*	21.66 ± 0.143	9.41 ± 0.07

Proximate composition of the curd

The proximate composition such as energy, protein, fat, carbohydrate, dietary fibre, moisture, ash, vitamin C, and calcium content of the developed chickpea curd was estimated with the AOAC method (2000). (Table 5) shows the proximate composition of the curd. The energy content of the control curd was found to be 86.31± 0.03 kcals/g comparatively lower than the sample (chickpea curd) which was 191± 0.81 kcals/g because the sample was prepared from chickpea. The carbohydrate content of the chickpea curd was found to be 12.11 ± 1.64g comparatively higher than the control 3.38 ± 0.03g. The carbohydrate content of cow's milk yogurt is 1.70% and for soy milk yogurt it was 4%. When compared with the other yogurts chickpea curd has higher carbohydrate content [6] [29]. The protein content of chickpea curd was found to be 35.1 ± 4.09 g which was comparatively higher than the control curd was found to be 7.41± 0.23 g. The higher protein content of the sample was due to the incorporation of chickpeas [7] [1]. The fat content of the control curd was found to be 4.81± 0.07 g comparatively higher than the sample (chickpea curd) which was 1.24 ± 0.02 g because chickpea is a plant-based product that is low in fat.

Table 5 Proximate composition of the curd

Parameters	Control	Sample	P-Value
Energy (Kcals)	86.31 ± 0.03	191 ± 0.81	0.01**
Carbohydrates (g)	3.38 ± 0.03	12.11 ± 1.64	0.01**
Protein (g)	7.41± 0.23	35.1 ± 4.09	0.02*
Fat (g)	4.81± 0.07	1.24 ± 0.02	0.20^{NS}
Fiber (g)	-	5.33 ± 0.03	0.28^{NS}
Ash (%w)	0.73± 0.07	0.66 ± 0.04	0.02*
Moisture (%w)	83.80± 0.00	84.3 ± 0.92	0.00**
Calcium (mg)	281.43± 0.01	49.04 ± 0.81	0.02*
Vitamin C (mg)	4.83±0.01	195.66 ± 4.18	0.06^{NS}

* – significant at 5% (p<0.05); ** - significant at 1% (p<0.01); NS – non-significant

The fiber content of the sample was 5.33 ± 0.03g calculated with mean score and standard deviation, taken in triplicates. The ash content represents the incombustible component remaining after a sample of the furnace oil is completely burned, the ash content of the chickpea curd sample was 0.66 ± 0.04% which showed slight variation compared with control curd 0.73 ± 0.07%. The moisture content of the chickpea curd sample was 84.3 ± 0.92% and the control was 83.80 ± 0.00%. The moisture content of the yogurt samples is affected by the changing temperature and longer storage period. The vitamin C content of the Sample was found to be 195.66 ± 4.18mg which was comparatively higher than the control curd which ranges about 4.83±0.01mg. The calcium content of the curd prepared from chickpea milk showed a lower amount when compared with the control. The calcium content present in the sample curd was 49.04 ± 0.81mg and the control was 281.43 ± 0.01mg given in the (Table 5). In a study done by [13], the calcium present in soy milk yogurt was 180 mg chickpea milk contains a negligible amount of calcium as it is plant-based milk, and it is also affected by the type of medium used for the starter culture to set the yogurt [20].

Table 6 Morphological and biochemical characterization

	Gram staining	Spore formation	KOH	Catalase	Glucose	Lactase	Cellobiose	Sucrose
LB1	Gram positive rod shaped	No spore formation	Negative	Negative	Positive gas production	Positive	Positive	Positive
LB2	Gram positive rod shaped	No spore formation	Negative	Negative	Positive gas production	Positive	Positive	Positive

In vitro probiotic efficacy

Isolation of lactic acid bacteria from curd

A total of 30 sample lactic acid bacteria were isolated from chickpea curd. Among them, 2 isolates were selected due to economic constraints and tested for biochemical characteristics and were found to be Gram-positive, endospore-negative, and catalase-negative.

Morphological and biochemical characterization

The present study reports gram-positive rod-shaped bacteria on MRS agar plates. The growth characteristic was observed at the temperature range of 15-45ºC. The gram-positive and rod-shaped isolates showed positive for glucose production, lactose test, sucrose test, and cellobiose test and they showed negative for the KOH test and catalase test [11].

In vitro characterization of probiotic properties

Tolerance to low pH

Out of the total 30 LAB isolates, 2 isolates survived pH 3.0 upon exposure for 12hours and 24hours hours. The survival rate of the isolates ranged from LB1 - 85.5% to LB2 - 90.8% at pH value (3) in 24-hour incubation periods. Probiotic microorganisms must survive in the GI tract at pH 3 or below, where food has to pass for 2–3 hours [8]. During the incubati0n period of 24 h LB2 showed analogous growth compared to Lb1 (Table 7).

Table 7 Tolerance to low pH

Isolates	pH	Count CFU/ml Initial (N_o)	Count CFU/ml Initial (N_1) 12 hrs	Survival rate in % (24 hrs)
LB1	3	235	201	85.5
LB2	3	217	197	90.8

Table 7A Tolerance to bile salts

Isolates	Bile salt %	Count CFU/ml Initial (N_o)	Count CFU/ml Initial (N_1)	Survival rate in % (24 hrs)
LB1	0.3	235	222	94.5
LB2	0.3	217	202	93

Tolerance to bile salts

Bile disrupts lipids and fatty acids of the cell membrane, which eventually decreases the survival rate of bacteria [32]. All of the 2 LAB isolates (LB1 and LB2) were able to survive above 90% in the presence of 0.3% of bile salt. Isolate LB1 was the most tolerant with a 94.5% survival rate followed by isolate LB2 with a 93% survival rate. The study done by [31] reports the viability of Lactobacillus at 0.3% bile, with a survival rate of 83.70% (Table 7A).

Bacterial adhesion to stainless steel plates

The adherence ability of the potential probiotic LAB isolates was found to range between 90.6% and 94%. Isolate LB1 showed the highest (90.6%) adherence rate followed by isolate LB2 with a 94% adherence rate [39] (Table 7B).

Table 7B Bacterial adhesion to stainless steel plates

Isolates	Count CFU/ml Initial (N_o)	Count CFU/ml Initial (N_1)	Adherence %
LB1	235	213	90.6
LB2	217	204	94

Water hydrophobicity

Hydrophobicity indicates bacterial adhesion to human intestinal cells which is a prerequisite for probiotic activity and considered an important selection criterion for potential probiotic strains (Soumitra *et al.* 2020). This hydrophobic nature of microbes is probably involved in the attachment of bacteria to epithelial tissue that confers bacterial maintenance in the human GI tract [37]. The water hydrophobicity ability of the potential probiotic LAB isolates was found to range between 84.59% and 80.83%. Isolate LB1 showed the highest (84.59%) hydrophobic rate followed by isolate LB2 with an 80.83% hydrophobic rate (Table 7C).

Table 7C Water hydrophobicity

	Initial OD	Final OD	Hydrophobicity %
LB1	0.448	0.069	84.59
LB2	0.433	0.083	80.83

Shelf-life study of the curd

Storage studies were carried out for the prepared sample and quality parameters were evaluated during storage. The

quality parameters of the sample produced were taken after the preparation and measurement were considered as the quality parameter on the 0th day. The quality parameters data were taken at regular intervals of 0th day, 1st day, 3rd day, and 7th day [38]. Glass storage extends the product's shelf life by up to 7 days. Because glass has a low rate of chemical reactions, the items within a glass bottle retain their freshness, aroma, and flavour. The curd prepared from chickpea milk was set in a pre-sterilized glass container and sealed with a lid. The formulated curd is then stored at 4° C in a refrigerator [29].

Table 8 Microbial changes during storage

Sample	*E. coli*, yeast and mould count				
	0th day	1st day	3rd day	5th day	7th day
	No colonies	No colonies	No colonies	No colonies	No colonies

Microbial changes during storage of the curd

The total yeast and mould count and E. coli count of the sample are given in (Table 8). Yeasts are eukaryotic organisms that are included in a group of organisms called – fungi, which also include moulds [26]. The absence of coliform yeast and moulds is an indication of efficient plant hygiene and sanitation during the storage period.

Sensory evaluation of the chickpea curd during the storage period

From above (Table 8A), we can conclude that there were slight changes during the storage period when compared to before the storage period. During 3rd day, there were no changes in appearance, colour, taste, texture flavour, and overall acceptability. During the 5th day, there were slight changes in the texture as the whey water started to accumulate on the top. On the 7th day, there were slight changes in the appearance, texture, and overall acceptability due to the accumulation of whey water on the top. The result of the shelf-life analysis of the chickpea curd can be concluded that the chickpea curd has retained its quality attributes throughout the shelf-life study.

Cost calculation of the curd

The total cost of the Chickpea curd was made on the basis of the cost of raw materials and labour costs. The raw material cost includes chickpeas. Processing cost includes electricity, labour charges, and miscellaneous cost. The nutritious and healthy chickpea curd prepared from chickpea milk costs a minimal charge. The cost of commercial dairy curd for 100ml was ₹50. Hence, when compared with the above study, the cost of 100ml of curd ₹10 is cost-effective and inclusive of all variable costs.

Table 8A Sensory evaluation of curd during storage

	Attributes	0th day	3rd day	5th day	7th day
Control	Appearance	7.4 ± 0.63	7.4 ± 0.63	7.3 ± 0.63	7.3 ± 0.63
	Colour	7.06 ± 0.70	7.06 ± 0.70	7.05 ± 0.70	7.04 ± 0.70
	Taste	7.33 ± 0.48	7.33 ± 0.48	7.33 ± 0.48	7.33 ± 0.48
	Texture	7.4 ± 0.73	7.3 ± 0.72	7.2 ± 0.72	7.0 ± 0.68
	Flavour	7.1 ± 0.98	7.09 ± 0.96	7.09 ± 0.96	7.06 ± 0.96
	Overall acceptability	7.8 ± 0.79	7.5 ± 0.77	7.2 ± 0.77	7.1 ± 0.77
Sample	Appearance	8.4 ± 0.50	8.4 ± 0.50	8.2 ± 0.49	8.1 ± 0.48
	Colour	8.66 ± 0.48	8.66 ± 0.48	8.66 ± 0.48	8.66 ± 0.48
	Taste	8.73 ± 0.45	8.73 ± 0.45	8.61 ± 0.40	8.60 ± 0.40
	Texture	8.53 ± 0.51	8.50 ± 0.49	8.49 ± 0.45	8.48 ± 0.45
	Flavour	8.73 ± 0.45	8.73 ± 0.45	8.7 ± 0.44	8.60 ± 0.40
	Overall acceptability	8.8 ± 0.41	8.8 ± 0.41	8.7 ± 0.39	8.6 ± 0.38

CONCLUSION

The present study of chickpea curd was found to be rich in energy, protein, fiber, and mineral-like vitamin C. It is also microbially safe for human consumption. The developed curd will not only improve nutritional status but also solve several nutritionally related problems in the community. Because there is a significant increase in lactose intolerance and gut-related problems among people. So instead of animal milk products, plant-based products, especially chickpea curd are an effective alternative with high nutritional value. The developed chickpea curd has the highest acceptance score. It was good in all physical properties like texture and colour attributes like dairy curd. Invitro probiotic efficacy was done on the developed chickpea curd which has good probiotic potential and the developed chickpea curd was also cost-effective when compared to the commercial curd. Nowadays people are more focused on health with good lifestyle choices and prefer the best and most economical products. Hence, the developed chickpea curd is more nutritionally significant than the commercial curd available in the market which can be suggested for gut strengthening, especially for the elderly population and gastrointestinal disorders too.

Ethical consideration

The current study was carried out after acquiring ethical approval from the Independent Human Ethical Committee (IHEC) on the following date: 01/10/2022 (Protocol No. SDNBVC/HSC/IHEC/2021/13), concluded by the Post Graduated Department of Food Science, Nutrition, and Dietetics, SDNB Vaishnav College for Women (Autonomous) Chromepet, Chennai.

Acknowledgement

The author extends sincere thanks to the PG Department of Food Science, Nutrition and Dietetics, SDNB Vaishnav College for Women, for providing the laboratory facilities and all other support.

LITERATURE CITED

1. Aguilera, Y., Dueñas, M., Estrella, I., Hernández, T., Benitez, V., Esteban, R. M., & Martín-Cabrejas, M. A. (2011). Phenolic Profile and Antioxidant Capacity of Chickpeas (Cicer arietinum L.) as Affected by a Dehydration Process. *Plant Foods for Human Nutrition*, *66*(2). https://doi.org/10.1007/s11130-011-0230-8
2. Aydar, E. F., Tutuncu, S., & Ozcelik, B. (2020). Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. In *Journal of Functional Foods* (Vol. 70). https://doi.org/10.1016/j.jff.2020.103975
3. Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic mechanisms of action. In *Annals of Nutrition and Metabolism* (Vol. 61, Issue 2). https://doi.org/10.1159/000342079
4. Cai, R., & Baik, B. K. (2001). Preparation of chickpea curd by heat-induced gelation. *Journal of Food Science*, *66*(9). https://doi.org/10.1111/j.1365-2621.2001.tb15204.x
5. Chen, X., Singh, M., Bhargava, K., & Ramanathan, R. (2018). Yogurt Fortification with Chickpea (Cicer arietinum) Flour: Physicochemical and Sensory Effects. *JAOCS, Journal of the American Oil Chemists' Society*, *95*(8). https://doi.org/10.1002/aocs.12102
6. Chukwu, M., & Onuh, E. F. (2020). Proximate Composition and Organoleptic Attributes of Legume-Yoghurt Samples Fermented by Lactic Acid Bacteria. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.3616418
7. Dasril, O., Putri, G. E., & Amar, S. (2019). Comparison of Nutritional Quality and Organoleptic of Dadih (Minangkabau Traditional Yogurt) of Cow Milk and Soy Milk as Functional Food Probiotics. *Global Journal of Medical Research: L Nutrition & Food Science*, *19*(1).
8. Dias, P. G. I., Sajiwani, J. W. A., & Rathnayaka, R. M. U. S. K. (2020). Consumer perception and sensory profile of probiotic yogurt with added sugar and reduced milk fat. *Heliyon*, *6*(7). https://doi.org/10.1016/j.heliyon.2020.e04328
9. Domagała, J. (2009). Instrumental texture, syneresis and microstructure of yoghurts prepared from goat, cow and sheep milk. *International Journal of Food Properties*, *12*(3). https://doi.org/10.1080/10942910801992934
10. Estifanos, H. (2014). Isolation and identification of probiotic lactic acid bacteria from curd and in vitro evaluation of its growth inhibition activities against pathogenic bacteria. *African Journal of Microbiology Research*, *8*(13). https://doi.org/10.5897/ajmr2014.66
11. Fatima, S. M., & Hekmat, S. (2020). Microbial and Sensory Analysis of Soy and Cow Milk-Based Yogurt as a Probiotic Matrix for Lactobacillus rhamnosus GR-1. *Fermentation*, *6*(3). https://doi.org/10.3390/FERMENTATION6030074
12. Flores-Silva, P. C., Berrios, J. D. J., Pan, J., Osorio-Díaz, P., & Bello-Pérez, L. A. (2014). Gluten-free spaghetti made with chickpea, unripe plantain and maize flours: Functional and chemical properties and starch digestibility. *International Journal of Food Science and Technology*, *49*(9). https://doi.org/10.1111/ijfs.12529
13. Fructuoso, I., Romão, B., Han, H., Raposo, A., Ariza-Montes, A., Araya-Castillo, L., & Zandonadi, R. P. (2021). An overview on nutritional aspects of plant-based beverages used as substitutes for cow's milk. In *Nutrients* (Vol. 13, Issue 8). https://doi.org/10.3390/nu13082650
14. Gamli, Ö. F., & Atasoy, A. F. (2018). Physico-chemical and sensorial properties of groundnut milk and it's yoghurt. *Journal of Food Measurement and Characterization*, *12*(3). https://doi.org/10.1007/s11694-018-9814-4
15. Heino, A. T., Uusi-Rauva, J. O., Rantamäki, P. R., & Tossavainen, O. (2007). of Dairy Technology 1364-727X Society of Dairy Technology. *International Journal of Dairy Technology IDT International Journal*, *60*(4).
16. Herrera A., C., & Gonzalez de Mejia, E. (2021). Feasibility of commercial breadmaking using chickpea as an ingredient: Functional properties and potential health benefits. In *Journal of Food Science* (Vol. 86, Issue 6). https://doi.org/10.1111/1750-3841.15759
17. Hirdyani, H. (2015). Nutritional composition of Chickpea (Cicerarietinum-l) and value added products. In *Indian Journal of Community Health* (Vol. 26).
18. Hussein, H., Awad, S., El-Sayed, I., & Ibrahim, A. (2020). Impact of chickpea as prebiotic, antioxidant and thickener agent of stirred bio-yoghurt. *Annals of Agricultural Sciences*, *65*(1). https://doi.org/10.1016/j.aoas.2020.03.001
19. Iyer, B. K., Singhal, R. S., & Ananthanarayan, L. (2013). Characterization and in vitro probiotic evaluation of lactic acid bacteria isolated from idli batter. *Journal of Food Science and Technology*, *50*(6). https://doi.org/10.1007/s13197-011-0445-6
20. J, S., & D, S. (2018). Procreation and Quality Analysis of Cow Curd by Using Starter Culture as Fermented Rice Rinsed Water. *Journal of Food Processing & Technology*, *9*(12). https://doi.org/10.4172/2157-7110.1000767
21. Joon, R., Mishra, K. S., Brar, G. S., Singh, P. K., & Panwar, H. (2017). Instrumental texture and syneresis analysis of yoghurt prepared from goat and cow milk. *The Pharma Innovation Journal*, *6*(7).
22. Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (Cicer arietinum L.): A review. *British Journal of Nutrition*, *108*(SUPPL. 1). https://doi.org/10.1017/S0007114512000797
23. Kamath, M. V., & Belavady, B. (1980). Unavailable carbohydrates of commonly consumed Indian foods. *Journal of the Science of Food and Agriculture*, *31*(2). https://doi.org/10.1002/jsfa.2740310211
24. Kim, S. E. un. (2015). Nutritional Screening and Assessment in Hospitalized Patients. In *The Korean journal of gastroenterology = Taehan Sohwagi Hakhoe chi* (Vol. 65, Issue 6). https://doi.org/10.4166/kjg.2015.65.6.336
25. Kosterina, V., Yakovleva, A., Koniaeva, V., & Iakovchenko, N. (2020). Development of formulation and technology of non-dairy soy-coconut yogurt. *Agronomy Research*, *18*(Special Issue 3). https://doi.org/10.15159/AR.20.107
26. Li, M., Dong, H., Wu, D., Chen, H., Qin, W., Liu, W., Yang, W., & Zhang, Q. (2020). Nutritional evaluation of whole soybean curd made from different soybean materials based on amino acid profiles. *Food Quality and Safety*, *4*(1). https://doi.org/10.1093/fqsafe/fyaa011
27. Lorea Baroja, M., Kirjavainen, P. V., Hekmat, S., & Reid, G. (2007). Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. *Clinical and Experimental Immunology*, *149*(3). https://doi.org/10.1111/j.1365-2249.2007.03434.x

28. Lourens-Hattingh, A., & Viljoen, B. C. (2001). Yogurt as probiotic carrier food. In *International Dairy Journal* (Vol. 11, Issues 1–2). https://doi.org/10.1016/S0958-6946(01)00036-X
29. Mawunyo Kwasi Fidelis, K., Ohene Emmanuel, A., Bediako Betty, A., & Kwesi Firibu, S. (2014). Nutritional and Sensory Characterization of Full Fat and Partially Defatted Peanut Soy Milk Yoghurt. *International Journal of Nutrition and Food Sciences*, *3*(3). https://doi.org/10.11648/j.ijnfs.20140303.19
30. Mulaw, G., Sisay Tessema, T., Muleta, D., & Tesfaye, A. (2020). Erratum: In Vitro Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products (International Journal of Microbiology, vol. 2019 DOI: 10.1155/2019/7179514). In *International Journal of Microbiology* (Vol. 2020). https://doi.org/10.1155/2020/6401356
31. Nath, S., Sikidar, J., Roy, M., & Deb, B. (2020). In vitro screening of probiotic properties of Lactobacillus plantarum isolated from fermented milk product. *Food Quality and Safety*, *4*(4). https://doi.org/10.1093/fqsafe/fyaa026
32. Niazi Amraii, H., Abtahi, H., Jafari, P., Mohajerani, H. R., Fakhroleslam, M. R., & Akbari, N. (2014). In vitro study of potentially probiotic lactic acid bacteria strains isolated from traditional dairy products. *Jundishapur Journal of Microbiology*, *7*(6). https://doi.org/10.5812/jjm.10168
33. Oladipo, I. C., Atolagbe, O. O., & Adetiba, T. M. (2014). Nutritional evaluation and microbiological analysis of yoghurt produced from full cream milk , tiger-nut milk , skimmed milk and fresh cow milk. *Pensee Journal*, *76*(4).
34. Papadimitriou, K., Zoumpopoulou, G., Foligné, B., Alexandraki, V., Kazou, M., Pot, B., & Tsakalidou, E. (2015). Discovering probiotic microorganisms: In vitro, in vivo, genetic and omics approaches. In *Frontiers in Microbiology* (Vol. 6, Issue FEB). https://doi.org/10.3389/fmicb.2015.00058
35. Parle, M., & Malik, J. (2014). CURD: A SEDATIVE WITH A BONUS BOWL OF USEFUL SIDE EFFECTS. *INTERNATIONAL RESEARCH JOURNAL OF PHARMACY*, *5*(3). https://doi.org/10.7897/2230-8407.050328
36. Parrish, C. R. (2018). Moo-ove Over, Cow's Milk: The Rise of Plant-Based Dairy Alternatives The Rise of Plant-Based Milks. *Practical Gastroenterology*.
37. Pratap, D., Maurya, V. K., Kumar, N., Singh, R., & Upadhyay, A. (2017). Studies on Physico-chemical and Organoleptic Properties of Soymilk Blended Dahi (Curd) with Toned Milk (Cattle Milk). *Current Nutrition & Food Science*, *14*(1). https://doi.org/10.2174/1573401313666170724112554
38. Samuella, K., & Kabba, K. (2017). Proximate and Microbial Profile of Couscous Yoghurt Produced from Soya Milk. *International Journal of Environment, Agriculture and Biotechnology*, *2*(4). https://doi.org/10.22161/ijeab/2.4.53
39. Shaikh, M., & Shah, G. (2013). Determination of Probiotic Properties of Lactic Acid Bacteria From Curd. *Global Journal of Biology, Agriculture & Health Sciences*, *2*(2).
40. Sharif, M. K., Rizwan Sharif, H., & Nasir, M. (2017). *Sensory Evaluation and Consumer Acceptability*. https://www.researchgate.net/publication/320466080
41. Sidhu, M. K., Lyu, F., Sharkie, T. P., Ajlouni, S., & Ranadheera, C. S. (2020). Probiotic yogurt fortified with chickpea flour: Physico-chemical properties and probiotic survival during storage and simulated gastrointestinal transit. *Foods*, *9*(9). https://doi.org/10.3390/foods9091144
42. Singh, G., Vij, R., & Katoch, S. (2021). Comparison of dairy milk with vegan milk of different types available in India. *~ 24 ~ The Pharma Innovation Journal*, *11*.
43. Stephen Ezeonu, C. (2016). Quantification of Physicochemical Components in Yoghurts from Coconut, Tiger Nut and Fresh Cow Milk. *Advances in Biotechnology & Microbiology*, *1*(5). https://doi.org/10.19080/aibm.2016.01.555573
42. Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., & Kopsahelis, N. (2019). Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. In *Nutrients* (Vol. 11, Issue 7). https://doi.org/10.3390/nu11071591
43. Thorning, T. K., Raben, A., Tholstrup, T., Soedamah-Muthu, S. S., Givens, I., & Astrup, A. (2016). Milk and dairy products: Good or bad for human health? An assessment of the totality of scientific evidence. In *Food and Nutrition Research* (Vol. 60). https://doi.org/10.3402/fnr.v60.32527
44. Vanga, S. K., & Raghavan, V. (2018). How well do plant based alternatives fare nutritionally compared to cow's milk? In *Journal of Food Science and Technology* (Vol. 55, Issue 1). https://doi.org/10.1007/s13197-017-2915-y