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Research Article

AMELIORATION IN THE NUTRITIONAL CONTENT OF CURD PREPARED USING PROBIOTIC YEAST *SACCHAROMYCES CEREVISIAE*ID18

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ABSTRACT

Dietary live yeast has been used as a fermenting agent in baking, distilling and brewing industries since ancient times. *Saccharomyces cerevisiae*Id18 isolated from traditional Indian fermented food-idli batter, exhibited probiotic attributes such as acid and bile salt tolerance, ability to grow at 37°C, resistance to commonly used antibiotics, auto-aggregationability and cell surface hydrophobicity. It showed antimicrobial action against enteric pathogens. It produced phytase, β-galactosidase, vitamin B_{12} and exopolysaccharides. It had the ability to assimilate cholesterol. This probiotic yeast was used as a starter to prepare curd; and it was also used to supplement traditional dairy starter to prepare the curd. On comparing the nutritional and sensory properties of probiotic curd with that of conventional curd, it was observed that the protein, vitamin B_{12} and glutamic acid levels doubled when probiotic yeast *S. cerevisiae*Id18 was used. The cholesterol levels in the probiotic curd were significantly reduced and glucose levels showed slight reduction. There was no significant decline in pH of the probiotic curd even after a week, indicating thereby less souring and prolonged shelf life. The viable counts of the probiotic yeast reduced slightly whereas the *Lactobacillus* counts declined considerably in the other curds, indicating thereby that the beneficial effects of probiotic curd were retained for a prolonged time period at 4°C. This indicates that on using the probiotic yeasts either alone or in combination with traditional dairy starter, the nutritional properties and the shelf life of the fermented dairy product were significantly improved.

Keywords: Probiotic, Dairy products, Yeast, *Saccharomyces cerevisiae,* Nutrition, Vitamin, Cholesterol.

1. INTRODUCTION

The preservation of food by fermentation is one of the oldest methods known to mankind. A typical example is of lactic acid fermentation, which is widely used for the preparation of several fermented milk products, such as curd (curd), yoghurt, acidophilus milk, shrikhand and various varieties of cheeses. Fermentation produces different intermediatory or end products, which give typical attributes to fermented milk [1]. The roles played by starters during fermentation of milks include-production of lactic acid, coagulation of protein and formation of gel, production of volatile flavour compounds like diacetyl, acetaldehyde etc., source of proteolytic and lipolytic enzymes, produce compounds like CO_2, alcohol, propionic acid, control the growth of pathogens and spoilage organisms. Some dietary cultures like *Lactobacillus acidophilus*, give health benefits and produces antibacterial substances [2]. Fermented milk products that are manufactured using starter cultures containing yeasts include yogurt, acidophilus-yeast milk, Kefir, Koumiss and Leban, cheese [3].

Curd is the most popular yoghurt-like fermented dairy product in the Indian sub-continent and consumed by most people almost every day with each meal either plain, sugared or salted [4]. The beneficial aspects of milk, curd and other dairy products are well documented [5, 6]. The chemical composition of curd has been reported as fat ranging from 5 to 8 %, protein 3.3 to 3.4 %, ash 0.75 to 0.79 % and lactic acid 0.5 to 1.1 %. During curd manufacture, maximum increase in folic acid, followed by riboflavin and thiamine were noted [7] thus, curd is considered a potential source of thiamine [8] and riboflavin [9]. Bhattacharay and Das [10] isolated from curd a few lactobacilli, which produced bacteriocins effective against human pathogens. Thus, the therapeutic properties of curd are well known, hence curd is used in several of Ayurvedic formulations (traditional Indian medicinal system)

[11, 12]. Curd is also used as a vehicle to incorporate probiotics into the health conscious consumers because of the fact that it is consumed by people daily [13].

A number of dairy starter cultures are widely used probiotics. Probiotics are combinations of live micro-organisms which, when administered in adequate amounts confers health benefits on the host. Probiotics are dietary supplements that contain beneficial micro-organisms such as bacteria or yeast, which helps in fighting off disease causing organisms and thus provide health benefits to humans. Nowadays probiotic organisms are increasingly incorporated into food as dietary adjuncts to help maintain a healthy microbial gastrointestinal balance, with possible resulting benefits for the human health [14]. Such microorganisms predominantly incorporated, into fermented dairy products, are the probiotic bacteria *Lactobacilli* and *Bifidobacteria* and yeast such as *Saccharomyces* spp. Most of the probiotic microorganisms belong to the genera of lactic acid producing bacteria, but some yeast strains that exist in dairy and fermented products are classified as probiotics [15]. However, to date, only *Saccharomyces cerevisiae* var. *boulardii* is considered a probiotic yeast because other "alternative" species need more definitive *in vitro* characterization before their utilization in human trials and applications [16, 17].

So far the active use of yeasts as dietary adjuncts for humans has been limited, despite the occurrences of yeasts as an integral part of the microflora of many dairy related products [18]. This may indicate that the potential of yeasts for incorporation into dairy products as probiotic agents has been overlooked [19]. There are reports on the beneficial effects of probiotic yeasts [20]. Several specific antagonistic interactions have been reported between culture yeasts like *Saccharomyces cerevisiae* and enteric pathogens, e.g. enteropathogenic *Esherichia coli, Shigella*_and *Salmonella*. It appears that *Saccharomyces cerevisiae* can survive passage though the intestinal tract, which further accentuates the possible use of yeasts as probiotics [21]. Out of the several yeasts isolated from fermented products, *Saccharomyces cerevisiae* according to EFSA (Europian Food Safety Authority) has a QPS (Qualified Presumption of Safety) status and is practically the only yeast commercialized as probiotic in human medicine [22].

This study reports the probiotic potential of *S. cerevisiae* Id 18 to improve the nutritional properties and shelf life of curd in comparison with the curd prepared by using traditional dairy starter; and commercial curd available in the market.

2. MATERIAL AND METHODS

All the chemicals used were of AR grade and procured from Qualigens, Himedia (Mumbai, India) and Sisco Research Laboratories (Mumbai, India).

2.1. Yeast Culture

A pure culture of *Saccharomyces cerevisiae* Id18 isolated from Idli batter was maintained on slants of MYPG (Malt extract-yeast extract-peptone-glucose) agar slants (gL^{-1}: malt extract 3.0, yeast extract 3.0, peptone 5.0, glucose 10.0 and agar 20.0, pH 5.6) and stored at 4°C [23, 24]. When required for experiments, it was activated by transferring to MYPG agar plates, incubated at 28°C for 48h.

2.2. Determination of Probiotic Properties

The probiotic attributes were determined as per standardized protocols [25].

2.3. Preparation of Fermented Dairy Products

The following combinations were used for preparing curd:

Milk + traditional dairy starter

Milk + traditional dairy starter + *S.cerevisiae*

Milk + *S.cerevisiae*

Commercial curd

100 mL of milk was taken in 5 separate flasks (250mL) and heated up to 85°C for 2 min and then cooled to 40°C. All the milk samples were aseptically dispensed into sterilized flasks, and then inoculated simultaneously at approximately the same level by using ainoculation level of 2% (v/v) to achieve an initial yeast count of $>10^8 cfumL^{-1}$ in the dairy products. In case of Sample 1 and 2, a half-tablespoon of traditional dairy curd was addedas a starter.

All the flasks were then incubated at 37°C for 18 h and after that kept at 4°C.

2.4. Nutritional Properties of Fermented dairy Products

2.4.1. Glucose Estimation

The curd samples were estimated for glucose concentration by using DNS reagent [26].

2.4.2. Protein Estimation

All the samples were then analyzed for protein concentration by Lowry's method [26].

2.4.3. Vitamin B_{12} Estimation

For vitamin B_{12} estimation, aliquots of supernatant of centrifuged curd samples were added to the basal medium containing all nutrients except that to be assayed. After inoculation with the assay organism *Lactobacillus lactis* subsp. *lactis*, and incubation at37°C for 18-24 h, the tubes were kept at 4°C to stop the growth and absorbance wasmeasured at 620 nm.

Vitamin concentration in food sample wascalculated as per the formula given

Vitamin = {Sample conc. (pgml^{-1}) X the appropriate dilution factor /(μg100g^{-1}) sample wt. (g)} X 100

2.4.4. Vitamin A Estimation

To 1 mL of the supernatant from centrifuged food samples, 4mL of Carr price reagent was added, in a semi dark room. Contents were mixed well and the absorbance was taken at 620 nm.

2.4.5. Glutamic Acid Estimation

To 2 mL of diluted food samples (with distilled water), 2 mL Ninhydrin reagent was added. The reagent was prepared by dissolving 0.5 g of ninhydrin in 30mL of acetone followed by 20 mL of acetate buffer of pH 5.5. The tubes were then kept in boiling water bath for 15 min and cooled to room temperature. Subsequently, 3 mL of 50% ethanol was added to each tube. The absorbance was measured at 570 nm.

2.4.6. Cholesterol Estimation

It was done by using Zlatkis method [27].

2.5. Physical Properties of the Fermented Dairy

2.5.1. pH

The pH of fermented food samples was determined at 0 day and 7 day.

2.5.2. Texture, Consistency and Aroma

The food samples were also analyzed for the texture, consistency and aroma by the project team.

2.5.3. Syneresis

20 g of curd from each food sample was centrifuged at 5000 rpm at 4°C for 10 min and the amount of whey (supernatant) was determined with the help of measuring cylinder [28].

2.6. Survival Rate of *S. cerevisiae* Id 18 and *Lactobacilli* in Curd Samples

Following fermentation, the curds were kept at 4°C. The viability of the probiotic yeast and *Lactobacilli* was determined at 0, 7 and 14 days using the method of serial dilutionand plating, on MYPG medium (for yeast) and MRS medium (for *Lactobacilli*). Inoculated plates were incubated at 30°C and 37°C for 24-48 h respectively.

All the experiments were conducted in triplicates and the mean values are presented.

3. RESULTS AND DISCUSSION

A comparison was made in the properties of the commercially available curd and the curd prepared from traditional dairy starter with the probiotic curd prepared by using *Saccharomyces cerevisiae*Id 18 isolate alone and *Saccharomyces cerevisiae*Id 18 blended with traditional dairy starter. The probiotic curds prepared in the study had low glucose levels of 25.8 and 23.7 mg g^{-1} respectively using yeast alone and in combination with a dairy starter, as compared to that 46 mg g^{-1} of commercial curd and 35.7 mg g^{-1} of curd prepared by using traditional dairy starter (Table 1). This shows that these probiotic curds can be consumed by diabetics without raising their blood glucose level. It was found that probiotic treatment had no effect on blood glucose levels in healthy rats, but it reduced glucose levels (up to 2-fold) in diabetic rats. The probiotic treatment of diabetic rats lowered blood glucose levels by insulin-independent mechanisms, suggesting that the administration of probiotics may be beneficial as adjunct therapy in the treatment of diabetes [29]. It can be concluded that fermented dairy product with low glucose levels, if consumed, can be helpful in lowering blood sugar levels. Inclusion of yeasts and yogurt cultures along with diacetyl producing starter cultures during the manufacture of curd is suggested for upgrading its dietetic properties.

Table 1: Comparison of nutritional properties of curd samples

sample	Glucose (mg/g)	Protein (mg/g)	Glutamic acid (mg/g)	Vit.B_{12} (μg/g)	Vit.A (μg/g)	Cholesterol (μg/g)
Milk+local curd	35.7	34.2	3.10	0.022	0.015	3510
Mlk+local curd+*S.cerevisiae*	23.7	78.5	6.25	0.095	0.014	1600
Milk+*S.cerevisiae*	25.8	110.6	8.5	0.13	0.016	1850

Commercial curd	46	4.0	4.0	0.032	0.150	3980

Curd prepared in the study by using this probiotic yeast Id18 had high protein values of 110.6 mg g^{-1} using *Saccharomyces cerevisiae*Id 18 alone and 78.5 mg g^{-1} when used in combination with the dairy starter; as compared to very low values of 34.2 mg g^{-1} in homemade curd and 4 mg g^{-1} *in* commercially available curd (Table 1). It is a well- known fact that *Saccharomyces cerevisiae* is a rich source of protein. They have been used successfully as a supplementary protein source in fish diet [30]. *Candida* sp., *Hansenula*sp., *Pichia* sp. and *Saccharomyces* sp. are of special importance as components in fish feeds. Recently, in animal feeding experiments, the use of supplemental yeast about 1 mg kg^{-1} has been found to yield various beneficial impacts [31] such as to compensate for the amino acid and vitamin deficiencies of cereals, and are recommended as a substitute for soybean oil in diets for fowl [32].

Yeasts are a source of vitamins, especially B-complex including B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6 (pyridoxine), B_9 (folic acid), and B_7 (biotin). These vitamins play an important role in various metabolic processes in the body such as in breaking down carbohydrates, fats, and proteins and provide the body with energy. Nutritional yeast grown on a molasses medium is an example of a food-based quasi-supplement approach that would provide a vegan source of vitamin B_{12}. One brand of nutritional yeast, Red Star T-6635+, has been tested and shown to contain active vitamin B_{12} [33]. In the present study, using probiotic yeast Id 18, the overall vitamin B_{12}content of fermented product significantly increased upto 0.13 μgg^{-1} as compared to 0.022 μgg^{-1} in the curd prepared from traditional dairy starter and 0.032 μgg^{-1} in the commercial curd (Table 1). Although the requirement for vitamin B_{12} is very low, but it is essential as it is involved as acofactor in a variety of enzymatic reactions and as a methyl donor in the synthesis of DNA and red blood cells. This vitamin is essential to maintain the integrity of the insulation sheath (myelin sheath) that surrounds the nerve cells [34]. A diet sufficient in vitamin B_{12} is essential to prevent severe pathologies, some of which are irreversible. It has been reported that vitamin B_{12} deficiency in the diet of pregnant female mice causes severe retardation of myelination in the nervous system and brain atrophy in the infants [35, 36]. There was no significant change in the vitamin A content in the fermented curd as compared to the curd prepared using conventional dairy starter.

It had been reported that yeast *Saccharomyces cerevisiae* can be used for production of glutamic acid [37]. The fermented curd prepared by using *S. cerevisiae* had a high glutamic acid level of 8.5 mg g^{-1} in comparison to the commercial curd and curd prepared from traditional dairy starter having 4.0 and 3.1mg g^{-1} respectively. Glutamic acid helps in the transportation of potassium into the spinal fluid. It also works as a neurotransmitter in the nervous system and helps in the transportation of the potassium across the brain. It is also used by the brain as a fuel. Its benefits help in curing diseases like epilepsy, ulcers, diabetes, mental retardation and Parkinson's disease [38].

In the present study, it was observed that the fermented curd prepared by *S. cerevisiae*Id18 had lower levels of cholesterol of 1850 μgg^{-1} and 1600 μgg^{-1} as compared to that of commercial curd and the curd prepared from traditional dairy starter, which had 3980μgg^{-1} and 3510 μgg^{-1} respectively (Table 1). *Saccharomyces cerevisiae* has previously been evaluated as a potential probiotic for assimilating cholesterol over the past few years [39]. As a feed additive, probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* has been reported to reduce serum cholesterol in animals [40, 41]. It has been recorded that even 1 % reduction in cholesterol can reduce the risk of cardiovascular diseases by 2-3 % [42], so the results of this investigation can be considered as promising as cholesterol reduction was shown to be up to 60% in this study.

Some studies report pH as a strong factor which influences viability of probiotic microorganisms in yogurt and other dairy products [43]. The pH of the rawmilk sample was 6.6 and upon fermentation, it decreased to 5.6. After keeping the curd for a week at 4°C, the pH of the probiotic curd, prepared using the yeast alone, decreased to 5.4 and for the curd prepared using yeast and dairy starter, pH decreased to 4.9. While the pH of the curd prepared from traditional dairy starter was decreased upto 4.6 (Table 2). This shows that the probiotic yeast does not cause much souring of the fermented food even after 7 days of preparation, showing thereby that it can be refrigerated for a week, without losing its flavour. When foods that are highly acidic are consumed they might cause symptoms like sour tummy, acid reflux and uncomfortable bloated state [44]. Due to the low pH of yogurt and the ability of yeasts to utilize organic acids, creates a selective environment for yeast growth [18].

As it is evident from Table 2, the texture and consistency of the curd prepared in the study was smooth and firm just as homemade/commercial curd when both traditional dairy starter and Id 18 isolate were used together and that of the curd made only using *Saccharomyces cerevisiae*Id 18 was less firm as investigated by project team. The aroma of fermented probiotic dairy products was curd like.

Amount of whey produced was 15mL in the curd prepared only by inoculating Id 18isolate in milk while in case of other thee samples it was 9mL. Whey contains various bioactive components that demonstrate a range of immune-enhancing properties [45]. Several studies have shown that whey-derived components can reduce the risk of metabolic syndrome, which can lead to various chronic diseases, such as cardiovascular disease and diabetes [46]. Clinical trials aimed at using whey in the treatment of cancers affecting the immune system have been successful [47]. Thus, whey provides health benefits to humans of all ages by providing specific bioactive components (above and beyond those necessary for nutrition) [48]. Its biological activities are partially attributable to specific bioactive peptides encoded in proteins [49].

Table 2: Sensory properties of curd samples and survival rate of *S. cerevisiae* Id18

Sample	pH		Texture/consistency	Aroma	Whey separation (ml)	Survival rate of *Lactobacilli* and *S.cerevisiae* respectively (cfu/mL)		
	1 day	7 days				1 day	7 days	14 days
Milk+local curd	5.6	4.6	Smooth & firm	Curd like/sweet	9	10^6	10^3	10
Milk+local curd +*S.cerevisiae*	5.6	4.9	Smooth & firm	Curd like/sweet	9	10^6+10^8	10^3+10^8	10+10^6
Milk+*S.cerevisiae*	5.6	5.4	Watery (cheese like)	Curd like/sweet	15	10^8	10^7	10^6
Commercial curd	5.2	4.9	Smooth &firm	Curd like/sweet	9	10^5	10^3	10

Several reports have shown that survival and viability of probiotic bacteria is often low in yoghurt and results in less than 10^8-10^9 cells/ml, which is daily recommended intake [50]. While contrary to this, in the present study, no decline in the viable count of the probiotic yeast (10^8cfu mL^{-1}) was observed after keeping the curd in refrigeration for a week. Even after two weeks of refrigeration the viable count of the probiotic yeast was as high as 10^6cfumL^{-1}. This proves that the probiotic curd has a prolonged shelf life as it contains high numbers of viable probiotic yeast. While, the viable count of the lactobacilli declined considerably in the home made curd and commercially available curd within a week of refrigeration (Table 2). It is essential that probiotic products sold with any health indication meet the minimum criterion of a million viable probiotic cells per milliliter product at the expiry date. According to literature, the minimum dosage of probiotic cells per day for any beneficial effect on the consumer is considered to be 10^8-10^9probiotic cfumL^{-1} [51, 52] corresponding to an intake of 100 g product containing 10^6-10^7cfu mL^{-1}per day. However, it has been reported [53] that *Lactobacillus acidophilus* and *B. bifidum*, present in bioyogurt, are unstable. Their poor survival in yogurt is attributed to the low pH of the environment and low acid-tolerance. Since yeasts have the ability to utilize organic acids, thereby increasing the pH of the environment, growth of probiotic yeast in association with probiotic bacteria has been suggested. In a study on growth and survival of probiotic in dairy products it was observed that the yeast species *Saccharomyces boulardii* is capable of utilizing the yogurt constituents as growth substrates and maintaining cell counts exceeding 10^6cfumL^{-1} which is in accordance with our yeast isolate having a cell count of10^8cfu mL^{-1}.

The frequent occurrences of yeast in dairy related products indicate the ability of yeasts to survive and metabolize milk constituents. The yeast isolate used in the present study is able to metabolize lactose (principle milk sugar) as it is β galactosidase producer. Lactose is broken down into glucose and galactose and then organism utilizes these components for its growth and survival. The major source for energy production in the yeast, *Saccharomyces cerevisiae*, is glucose and glycolysis is the general pathway forconversion of glucose to pyruvate, whereby production of energy in form of ATP iscoupled to the generation of intermediates and reducing power in form of NADH forbiosynthetic pathways [54].

4. CONCLUSIONS

A high quality probiotic curd using a novel strain *S. cerevisiae*Id18 was successfully developed, which has increased levels of protein, vitamin B_{12}, glutamic acid; and lowered levels of glucose and cholesterol; without deteriorating the quality and aroma. This probiotic curd can be stored in refrigeration for long because of the prolonged survival and maintenance of viable yeast count, along with lesser decline in pH. From the results of this study on the preparation of fermented dairy product, it is evident that *Saccharomyces cerevisiae*Id 18can be used as a good alternative or as a supplement to conventional dairy starter for the preparation of probiotic curd.

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Conflict of interest

None declared

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