RESEARCH ARTICLE

Improved version of curd prepared by adding exopolysaccharides produced from a probiotic *Lactobacillus paraplantarum* KM1

Kanika Sharma and Nivedita Sharma

Received: 06 October 2018 / Accepted: 17 December 2018 / Published online: 18 April 2019
© Indian Dairy Association (India) 2019

Abstract : In the present study, sensorial, microbiological and physicochemical difference of three sets of curd were studied, as well as the changes taking place during storage at 4°C for 7 days. The 1st set of curd was produced with curd starter culture and exopolysaccharide (EPS) producing from lactic acid bacterial strain i.e. *Lactobacillus paraplantarum* KM1, the second set with curd starter culture with 0.2% (w/v) purified EPS i.e. producing from *L. paraplantarum* KM1 and the third with just curd starter culture (as control curd). Many changes were observed in terms of microbial evaluation; for all three set of curd, the viable count i.e. in log cfu/ml of curd increased till 3rd day i.e. for set A (13.05), set B (10.2) and set B (9.5) but after 3rd it showed a decrease in number, after 5th day significant difference occur between all three treatments during storage period at 4°C till 7 days. A decrease in pH followed by an increase in lactic acid was observed during storage for all three set of curd, which was mostly attributed to the activity of the curd starter culture. Curd made with purified EPS had significantly ($P < 0.05$) lower firmness, low syneresis and higher viscosity values than control curd. According to the results, exopolysaccharide enhance viscosity, texture and mouth-feel and to avoid syneresis in curd. The results of this study suggest that the use of purified EPS could provide better textures for curd than those imparted by other chemical additives.

Keywords: Purified EPS, *L. paraplantarum* KM1, Microbial and sensorial evaluation, Viscosity

Introduction

Exopolysaccharides (EPS) producing lactic acid bacteria (LAB) are widely used for the manufacture of different dairy products to improve its texture and reduce syneresis without addition of hydrocolloids. During milk fermentation in curd manufacture, the pH decreases as the lactic acid is produced by the native culture. Casein begins to aggregate at pH 4.7, isoelectric point, forming a fragile gel net so, at the end of fermentation; the gel of the set-type curd is usually broken to produce stirred curd and the subsequent operations of mixing, pumping and packaging impact in its structure, decreasing the apparent viscosity. Many chemical additives are proposed to increase firmness and avoid syneresis: stabilizers, hydrocolloids (Keogh and O’Kennedy, 1998), gelatin, whey protein or calcium (Supavititpatana *et al.*, 2008) but with the addition purified exopolysaccharide (EPS) in relatively small quantity, 40 to 400 mg/L (De Vuyst *et al.*, 2003; Sharma *et al.*, 2017) also give better impact on curd by means of rheology, sensorial or microbial evaluation. A very little is known and few studies has been carried out to influence interaction between mil constituents and this natural thickener. So, the objectives of the present study are a trial to improve the physicochemical, rheological, microbiological and sensory properties of fermented milk, by using purified EPS as well as EPS-producing probiotic strains.

Materials and methods

Bacterial strains and culture conditions

Lactobacillus paraplantarum KM1 strain with NCBI accession number KX 671558 was isolated from human mother milk of Himachal Pradesh and maintained at -80°C in refrigerator. The growth MRS medium medium used for the production of EPSs producing lactic acid bacterial strain and was carried out at 37°C for 24 h with a 10⁸CFU/ ml inoculum.

Effect of purified EPS on curd

Kanika Sharma (✉) and Nivedita Sharma
Microbiology Research Laboratory, Department of Basic Sciences,
Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan-
173230, HP, India
E-mail: kanikasharma1994@gmail.com

Recipe

For curd preparation, whole milk was procured from local market. 500 ml milk was heated at 85°C for 15 min and was cooled down to 40°C. Pasteurized milk was inoculated with starter culture (curd inoculum). Set A was inoculated with EPS producing bacteria *L. paraplantarum* KM1 at the rate of 10⁸ CFU/ml and Set B was inoculated with 0.2% (w/v) purified exopolysaccharide from *L. paraplantarum* KM1 while control was the starter culture without supplemented with EPS producing bacteria and purified EPS. Fermentation was allowed for 6 h at 35°C followed by storage at low temperature 4°C (Fig. 1)

Sensorial evaluation

Sensorial evaluation of each sample was done in terms of appearance, texture, flavor and overall acceptability. Nine point hedonic scale method as given by Larmond, (1982) was allowed for conducting the sensory evaluation of probiotic and EPS evaluated food product.

Microbial evaluation during storage

The colony count was observed during storage period by standard spread plate count method. MRS agar was used to enumerate lactic acid bacteria while nutrient agar was used to enumerate aerobic mesophilic bacteria and malt extract agar used for mold count (Handa and Sharma, 2017; Sharma *et al.*, 2017).

Water- holding capacity (Doleyres and Lacroix, 2005)

Water- holding capacity (WHC) capacity was determined by Doleyres *et al.* (2005). 15g of curd was centrifuged at 5000 rpm at 4°C for 10 min. Whey expelled was removed and weighed. WHC was calculated as given below

$$\text{WHC (\%)} = (M1/M2) \times 100$$

M1: Mass of precipitate after centrifugation

M2: Mass of sample

Acidity in terms of lactic acid (Ranganna, 1997)

An aliquot of the sample prepared was diluted with recently boiled distilled water. Phenolphthalein solution (1% v/v-2-3 drops) used as an indicator and titration was done with 0.1N NaOH. Titer value was noted and calculations were done as percent anhydrous lactic acid.

$$\text{Titration acidity (\%)} = \frac{\text{Titer} \times \text{Normality of alkali} \times \text{volume made up} \times \text{equivalent weight}}{\text{Volume of sample taken} \times \text{volume of aliquot taken} \times 100} \times 100$$

pH determination

pH of sample was measured using pH meter.

Total soluble solids (Ranganna, 1997)

TSS was measured by diluting sample (100 times) on the prism of a hand refractometer. The results were expressed as °B.

Viscosity (Ranganna, 1997)

Ostwald viscometer was used to determine the relative viscosity of the curd. It was expressed a time (in sec) required for emptying (flowing out) a definite volume of the sample compared to time (in sec) taken by distilled water under identical conditions of temperature and volume.

Results and discussion

To examine the effect of exopolysaccharide (EPS) on the texture, physicochemical characteristics, firmness, viscosity and sensory quality of curd, purified EPS was added to milk produced from a *L. paraplantarum* KM1 potential probiotic strains along with native inoculum as a starter culture.

Curd

In the present investigation an attempt has been made to prepare EPS based curd. The curd was prepared using cow milk and fermenting it with native starter inoculum. The curd prepared in the present study was divided into three sets; Set-A was inoculated with *L. paraplantarum* KM1+ Native inoculum (Starter culture: Probiotic culture = 1:5 @ 10⁸cfu/ml) while in Set-B, purified EPS from *L. paraplantarum* KM1+ native inoculum. Set-C was taken as control enriched only with native inoculums (10⁸cfu/ml) (Fig 2). Each set was carried out for fermentation at 37°C for 4h. The fermentation was terminated by keeping these sets at low temperature i.e. 4°C and all these sets were subjected for further evaluation. Physicochemical and microbiological analysis of each prepared set of curd was performed.

Physico-chemical properties of curd

Prepared curd samples were kept under refrigeration and the quality attributes (pH, total soluble solids, titrable acidity, water holding capacity and viscosity) were examined.

Water- holding capacity (WHC)

WHC measurements were showed significant differences between set A, set B and set C (control) of curd samples. WHC of set A and set C (control) were 53.2% and 48.5 % respectively and this value increased to 63.4% when 0.2% EPS was added. The water holding capacity of set B was good as compared to control (Table 1). This result confirmed that EPS was able to improve

WHC of the curd by interacting with proteins and micelles (Hassan, 2008).

pH and titrable acidity

The changes in pH and titrable acidity of set A, set B and set C of curd were shown in Table 1. It was observed that the pH value slightly decreased in set A (3.9) rather than set B and set C (4.2 and 4.0). Titrable acidity of set B was minimum i.e. 0.56 than set A (0.67) and set C (0.78).

Viscosity

Viscosity of curd of each set was different from each other. By adding 0.2% purified EPS in curd had the highest value of 4.36 centi Poise(CPS) in set B than set A and set C i.e. 3.67 and 3.60 respectively (Table 1). It clearly had shown that the purified EPS could improve the viscosity of curd directly (Zhang *et al.*, 2010).

Total-soluble solids (TSS)

Curd stability is one of the most important physical properties. The TSS content of curd varied increasingly with increasing levels of EPS. In the present study, the maximum TSS was observed in set B i.e. 20.7⁰B while 19.0⁰B and 19.5⁰B were observed in set C (control)

Fig 1 Schematic form of curd formulation by using purified EPS from *L. paraplantarum*KM1

Fig 2 a) Morphology of *L. paraplantarum* KM1 b) Purified EPS *L. paraplantarum* KM1

Fig 3 Curd formation by using purified exopolysaccharides from *Lactobacillus paraplantarum* KM1

and set A respectively (Table 1). Thus set B showed overall improved qualities of desirable traits of good quality curd proving the effectiveness of purified EPS added as compared to direct addition of EPS proving cells and control.

Sensorial evaluation

Freshly prepared curd samples were assessed by 10 panelist using a 9 point sensory hedonic scale for some sensory parameters (viz. appearance/colour, flavour, texture, taste and

overall acceptability), as described by (Amerine and Joslyn, 1965). In a sensory evaluation curd Set C (7.0) was least accepted whereas curd Set B had a maximum acceptability as it scored 7.98 out of 10 (Fig 4) and set A showed 7.4 score. The results showed significantly highest acceptable effect of set B based on sensory attributes of curd compared to control. The results of above experiment also indicated that the purified EPS from *L. paraplantarum* KM1 contributed a significant influence on the overall acceptability of the product and presence of EPS in curd

Fig 4 Sensorial evaluation of exopolysaccharides based probiotic curd

Fig 5 Total viable count of exopolysaccharide based curd

resulted in better product characteristics in terms of color, mouth solubility, creamy taste effects positively.

Microbiological evaluation

The microbiological evaluation of curd was carried out to validate predicted growth values during storage period. Fig 5 revealed the data regarding viable colonies in terms of log cfu/ml. The viable colonies of each treatment were enumerated on 0 day, 3rd, 5th and 7th day of fermentation. Many changes were observed in survival of LAB, other bacteria (*Bacillus etc.*) and molds of curd made with non EPS set A (control), EPS producing LAB (set A) and with purified EPS (set B) during storage period at 4°C for 7 days. It was observed that at 0 day cfu/ml present in set A were (10.33), set B (7.0) and minimum in set C (7.63). The viable counts in curd increased till 3rd day i.e. for set A (13.05), set B

(10.2) and set B (9.5) but after 3rd it showed a decrease, but decreased after 5th with significant differences between all treatments (Fig 5). The viable count were higher in almost fresh samples than during the end of storage period. Viable count became gradually decrease for set A on 7th day with log cfu/ml (6.4), set B with log cfu/ml (4.9) and minimum for set C log cfu/ml (4.3) for lactic acid bacteria.

Water-holding capacity (WHC)

The gel system with EPS addition forms a three dimensional network structure that has good ability to retain moisture because of the interaction between milk proteins and EPS. However, the pores among the structure start bearing relatively loose with the increasing amounts of EPS addition. Perhaps EPS can also decrease the syneresis in curd and improve the stability of dairy

products (Duboc and Mollet, 2001; Prasanna *et al.*, 2012). Yang *et al.* (2013) showed WHC measurements significant differences between EPS fortified and control yoghurt samples. WHC of the control yoghurt was 45%, and this value increased to 57% when 0.1% EPS was added. With increasing EPS addition, WHC of buffalo yoghurt decreased slightly and changed to 49% when 3% EPS was added. No significant difference was found between 0.2% EPS and 0.3% EPS groups.

pH and titrable acidity

Titration acidity was measured in terms of lactic acid. Since, titration acidity is negatively correlated with pH, which means while pH decreased, titration acidity increased. pH is lower down due to remaining microbial activity that results in continuous lactose fermentation. Lactose present in milk is continuously converted to lactic acid by lactic acid bacteria, which lowers down the pH (Iyer *et al.*, 2006). Low pH not only contributing to the texture of curd but it ionizes calcium and thus facilitate its absorption in intestine. Acidic pH also reduces the inhibitory effect of phytic acid on calcium bioavailability and improve lactose absorption. Guven and Karaca (2002) also found that the titration acidity and pH values of the set type yoghurt added with 0.1-0.3 g inulin / 100 ml milk were not significantly different possibly due to small changes in activity of curd. The ideal pH of the EPS based finished product of yogurt should be between 4 to 4.1. The decrease in electrostatic repulsion between casein molecules occurs at pH 4.2; as the net negative charge on the casein decreases. When the pH of milk becomes close to the isoelectric point (pH 4.2) then casein-casein attractions increase due to increased hydrophobic and electrostatic charge interactions. The three dimensional network is formed consisting of clusters and chains of caseins due to the acidification process.

Viscosity

The increased viscosity shows interaction between EPS and proteins, which form a dense network with a higher elasticity because of Van-der Waals force and electrostatic repulsion. The three dimensional structure of the gels play a key role in reducing the external force and thus becomes able to maintain the textural property of curd. Li *et al.* (2014) showed that the viscosity of yoghurt had significantly higher apparent viscosity (4.5 CPS) by adding 0.3% purified EPS as compared to control (non-EPS) (3.4 CPS).

Total-soluble solids (TSS)

The chemical composition of the milk base especially total solids has the major effect on the acceptability of concentrated curd. Concentrated curd containing < 20°B total solid has been assessed as “thin and tasteless” and that with > 25°B total solid becomes gummy and bitter (Güven and Karaca, 2002.) while, TSS lies between 20 to 25°B is assessed as smooth, firmness and show its stable and thick form. Karasu *et al.* (2015) also reported that addition of EPS resulted in higher TSS contents in curd rather. Thus set B overall and improved qualities the desirable traits of good quality curd proving the effectiveness of purified EPS added as compared to direct addition of EPS proving cells and control.

Sensorial evaluation

Given that stabilizers are indispensable ingredients in dairy products of high rheology; it should be very important for dairy industry which would aim to save costs for stabilizers that are readily used in the production of curd. In literature study, the addition of EPS (0.2-0.3 g/100 ml) significantly affected appearance, color, taste, texture and overall preference. Good quality curd should maintain strong curd integrity without any sign shrinkage and disintegration into lumps and whey-off. It should also possess pleasant odor and flavor and especially set the curd, which is also relates to the appearance and mouth-feel, can positively affect of acceptability or preference of consumers. Ibrahim, (2015) showed that sensory quality acceptance of curd, varies significantly in curd with EPS or without EPS; the curd formulation with EPS was smooth and without syneresis. However, the curd made with non EPS (control) had significantly lower rating for body and texture, appearance and color, flavor and overall acceptance score due to wheying-off on the curd surface.

Sensory quality evaluation for the curd was done using a descriptive test by Karasu *et al.* (2015). The descriptive test was done using panelists from the study body. The sensory quality attributes under consideration were smell, mouth feel and taste and appropriate descriptions were used. For smell and taste, a well defined key used with numbers from 1-5 numbers with 1 being poor and 5 excellent. For mouth feel or consistency, the key used had 1-5 numbers with 1 being thin and 5 being extremely thick with purified EPS.

Microbiological evaluation

Table 1 Physicochemical characteristic of curd

Samples	Water-holding capacity (%)	pH	Titration acidity	Viscosity of curd (CPS)	TSS(°B)
Control*	48.58	4.0	0.78	3.60	19.00
Set A**	53.24	3.9	0.67	3.67	19.50
Set B ***	63.42	4.2	0.56	4.36	20.70
Mean	55.08	4.03	0.67	3.87	19.73
CD	(0.99)	(0.08)	(0.02)	(0.07)	(0.43)

In addition to lactic acid bacteria, total aerobic mesophilic bacteria, yeast and mold were also enumerated and it was observed that total aerobic mesophilic bacteria, yeast and mold were below the detection limit ($<10 \log \text{CFU/ml}$) as shown in Fig 5. Data obtained from analysis of the samples were evaluated by variance of analysis and the differences among means were calculated. Statistically it had been confirmed that there was non significant change occurred in viability of microbial cells during storage and set A contains the highest number of beneficial LAB compared to control i.e. set C and B. It may be concluded that curd formation by using purified EPS *L. paraplantarum* KM1 was found effectively active with good water holding capacity, texture, rheology, smoothness, mouth feel and improve taste as well as good probiotic cumulative score.

Conclusions

This is the first study that systematically examined the physicochemical, rheological, sensorial and microbial properties of curd produced by using purified EPS. Purified EPS reduced syneresis and increased the firmness of curd. The curd prepared with addition of purified as well as EPS producing *L. paraplantarum* KM1 showed a well defined porous gel structure. The EPS of the lactobacillus most likely interacted with the milk proteins and changes in the viscosity and water holding capacity of curd were observed during storage. Overall, this study showed that the purified EPS is from potentially probiotic strain with improvement of physicochemical and rheological properties. However, the acceptance of new dairy products by the consumers is governed mainly by its appearance and mouthfeel. Therefore, a sensory evaluation of the produced curd will be carried out to assess its consumer acceptability

Acknowledgements

The authors are very thankful to the Department of Environment, Science and Technology, HP Govt. India for fulfillment of this study.

References

- Amerine MA, Joslyn MA (1965) Sensory examination of wines: desert, appetizer and related flavored wines; the technology of their production. University of California, Division of Agricultural Sciences 357-371
- Broadbent JR, McMahon DB, Welker DL, Oberg CL, Moineau S (2003) Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: A review. Journal of Dairy Science 86: 407-423
- Damin, M R, Minowa E, Alcantara MR, Oliveira MN (2008) Effect of cold storage on culture viability and some rheological properties of fermented Milk prepared with yogurt and probiotic bacteria. Journal of Texture Studies 39: 40-55
- De Vuyst LM, Zamfir F, Mozzi T, Adriany V, Marshall B, Degeest, Vaningelgem (2003) Exopolysaccharide producing *Streptococcus thermophilus* strains as functional starter cultures in the production of fermented milks. International Dairy Journal 13: 707-717
- Doleyres Y, Lacroix C (2005) Technologies with free and immobilised cells for probiotic bifidobacteria production and protection. International Dairy Journal 15: 973-988
- Duboc P, Mollet B (2001) Applications of exopolysaccharides in the dairy industry. International Dairy Journal 11: 759-768
- Folkenberg DM, Dejmek P, Skriver A, Ipsen R (2005) Relation between sensory texture properties and exopolysaccharide distribution in set and in stirred yoghurts produced with different starter cultures. Journal of Texture Study 36: 174-189
- Güven M, Karaca OB (2002) The effects of varying sugar content and fruit concentration on the physical properties of vanilla and fruit ice cream type frozen yoghurts. International Journal of dairy technology 55: 27-31
- Handa S, Sharma S (2017) In vitro probiotic potential of *Lactobacillus delbreuckii* subsp. *Bulgaricus* F18 isolated from homemade butter. Microbiology 86: 143-149
- Hassan AN (2008) ADSA foundation scholar award: possibilities and challenges of exopolysaccharide-producing lactic cultures in dairy foods. Journal of Dairy Science 91: 1282-1298
- Hassan AN, Frank JF, Schmid KA, Shalabi SI (1996) Textural properties of yogurt made with encapsulated non-ropy lactic cultures. Journal of Dairy Science 79: 2098-2103
- Ibrahim HA (2015) Effects of exopolysaccharide producing starter cultures on physiological, rheological and sensory properties of fermented camel milk. Emirates Journal food agricultural 27: 374-383
- Iyer A, Mody K, Jha B (2006) Emulsifying properties of a marine bacterial exopolysaccharides. Enzyme Microbiology Technology 38: 220-222
- Karasu S, Toker OS, Yilmaz MT, Dertli E (2015) Thermal loop test to determine structural changes and thermal stability of creamed honey: Rheological characterization. Journal of Food Engineering 150: 90-98
- Keogh MK, O'Kennedy B (1998) Rheology of stirred yogurt as affected by added milk fat, protein and hydrocolloids. Journal of Food Science 63: 108-112
- Larmond E (1982) Laboratory methods for sensory evaluation of food. Publication No. 1637. Ottawa: Food Research Institute, Canada Department of Agriculture
- Li W, Ji J, Tang W, Rui X, Chen X, Jiang M, Dong M (2014) Characterization of an antiproliferative exopolysaccharide from *Lactobacillus helveticus* MB2-1. Carbohydrate Polymer 105: 334-340
- Prasanna PHP, Grandison AS, Charalampopoulos D (2012) Screening human intestinal *Bifidobacterium* strains for growth, acidification, EPS production and viscosity potential in low-fat milk. International Dairy Journal 23: 36-44
- Ranganna S (1997) Handbook of analysis and quality control for fruit and vegetable products, 2nd Edn. Tata McGraw Hill Publishing Company Ltd. New Delhi India 1109
- Sharma K, Sharma N, Sharma R (2017) An evaluation of In-vitro potential of novel *Lactobacillus paraplantarum* KM0 (KX671558) strain isolated from milk. Proceedings of the Indian National Science Academy 83: 689-699
- Sharma S, Sharma N, Handa S, Pathania S (2017) Evaluation of health potential of nutritionally enriched Kodo millet grown in Himachal Pradesh, India. Food Chemistry 214: 162-168
- Supavititpatana P, Wirjantoro A, Apichartsrangkoon, Raviyan P (2008) Addition of gelatin enhanced gelation of corn- milk yogurt. Food Chemistry 106: 211-216
- Tamime YA, Robinson RK (2007) Yoghurt: Science and technology (3rd ed.). Boca Raton, FL, USA: CRC
- Zhang H, Vanderleyden J, De Keersmaecker SCJ (2010) Exopolysaccharides of *Lactobacillus rhamnosus* GG form a protective shield against innate immune factors in the intestine. Microbiology Biotechnology 7: 1-7