RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.4.1035-1041

Effect of exopolysaccharide producing starter culture on the characteristics of skim milk coagulum

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Citation: Amrutha T.A. and Beena A.K. 2023. Effect of exopolysaccharide producing starter culture on the characteristics of skim milk coagulum. *J. Vet. Anim. Sci.* **54**(4):1035-1041
DOI: https://doi.org/10.51966/jvas.2023.54.4.1035-1041

Received: 13.06.2023 *Accepted: 15.11.2023* *Published: 31.12.2023*

Abstract

*The present study attempted to evaluate the effect of incorporating an exopolysaccharide (EPS) producing indigenous strain of lactic acid bacteria (LAB) with a non-EPS producing industrial strain of LAB on the physico-textural properties of product. For this, characteristics of the skim milk coagulum prepared using monocultures and mixed cultures were determined and compared. EPS producing Pediococcus pentosaceus DMG01as well as the non-EPS producer Lactococcus lactis were graded as weak acid producer based on Horrall Elliker test. Ability of the isolate DMG01 to ferment citrate endorsed its flavour production potential. Colony characteristics in Congo red agar indicated the indigenous isolate to be an EPS producer and industrial stain a non-EPS producer. The treatment products were prepared using EPS negative LAB (A), EPS producing P.pentosaceus (B) and a combination of these in the ratio 1:1 (C). The titratable acidity of curd B (0.96 percent LA) and curd A (*0.86 *per cent LA) was significantly less than C (1.10 per cent LA). In line with the titratable acidity, pH differed significantly between the treatments. Syneresis value of A (1.72 per cent) was significantly higher than both B (1.55 per cent) and C (1.28 per cent). Penetrometer reading was also significantly higher for A (36.25mm), indicating that the curd tension of the A was significantly lower than both B and C. Scanning electron microscopy revealed extensive fusion of casein micelles in milk coagulum prepared with EPS producing starter culture. The results underscore the advantageous effect of EPS producing starters on textural characteristics of the fermented product.*

Keywords*: Pediococcus pentosaceus DMG01, exopolysaccharide, scanning electron microscopy*

The quality of the fermented milk products, especially its techno-textural properties very much relies on the type of starter cultures. The potential for rapid acidification of the raw material

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was the primary criterion often assessed for choosing starters. Recently, there is a shift in the selection criteria and researchers are now focusing on the ability of starters to produce functional biomolecules that could confer additional technological and health benefits for the products. Nowadays, the health-conscious population prefers low calories especially low-fat products. But the removal of fat often results in textural and functional defects like a weak body, poor texture, and lack of proper flavour in the case of fermented milk products (Costa *et al*., 2010; Costa *et al*., 2012). Exopolysaccharides (EPSs) are high-molecular-weight polymers that are composed of sugar residues and are secreted by microorganisms into the surrounding environment (Poli *et al.,* 2011). Exopolysaccharides produced by lactic acid bacteria (LAB) are commonly used in the dairy industry to improve the texture and viscosity of fermented milk, to reduce syneresis, as well as to improve yield. As a hydrocolloid, EPS exhibit viscoelastic properties that could influence the textural and functional properties of fermented dairy products. Consumer acceptance of fermented dairy products depends mainly on their physical properties such as water-binding capacity, texture, and rheology. Exopolysaccharide, a postbiotic functional bioactive ingredient, produced in a matrix during fermentation, is also acclaimed to be a booster for a healthy gut (Behare *et al*., 2009; Shukla and Goyal, 2014; Juvonen *et al.,* 2015). The present study attempted to evaluate the effect of incorporating an EPS-producing indigenous strain of lactic acid bacteria (LAB) with a non-EPS-producing industrial strain of LAB on the physical-textural properties of the product.

Materials and methods

Strains

An indigenous goat milk isolate of EPS-producing strain *Pediococcus pentosaceus* DMG01 (NCBI accession no MK368400), was obtained from culture stock of Department of Dairy Microbiology, VKIDFT, Mannuthy and *Lactococcus lactis* (CHN-II-non EPS producer) was purchased from CHR-Hansen.

Congo red assay

EPS production potential was evaluated based on colony characteristics when streaked on Congo red agar (Freeman *et al.,* 1989). Congo red agar was prepared by adding 0.1 percent Congo red solution at a level of nine percent to Brain Heart Infusion agar containing five percent sucrose. The colonies were streaked on Congo red agar and incubated at 37 ^{0}C. The formation of slimy and shiny black colonies within 24h of incubation was suggestive of EPS production.

Citrate utilisation

The citrate utilisation potential of the culture was assessed in a media designed by Kempler and McKay (1980). The isolate was streaked on the Kempler's media and incubated at 37 ^{0}C/ 48 h. Formation of bluish green colonies was taken as citrate positive.

Determination of the activity of cultures

The isolates to be evaluated (*Lactococcus* culture CHN-II, *Pediococcus pentosaceus* and their combination in the ratio 1:1) were inoculated at a concentration of three percent in sterilised skim milk, incubated at 37 °C for three and half hours. The developed acidity was determined by titrating with 0.1 N NaOH to a faint pink colour using phenolphthalein as an indicator. The results were recorded as per cent lactic acid and expressed as the rate of acid production of the culture. The cultures exhibiting a developed acidity of 0.4 per cent lactic acid were graded as active culture; those with developed acidity in the range 0.3 to 0.35 percent LA were graded as slow active cultures. If less than 0.3 percent LA, culture was considered to be unfit for preparation of products (Horrall and Elliker, 1950).

Skim milk coagulum characteristics

The samples were prepared using sterilised skim milk (12.5% TS) to make sure that background flora do not contribute to the product characteristics. Physico-chemical characteristics (pH, titratable acidity, syneresis and curd tension) and microstructure of the skim milk coagulum were prepared using the monocultures of *L. lactis* (A), non-EPS producer and *P. pentosaceus* (B), EPS producer. Rate of inoculum was two per cent and incubation was done at 37 °C for 18 h. The third treatment sample (C) was prepared using mixed starter in the ratio 1:1 at a level of two percentage. Incubation conditions followed was same as that of monoculture.

pH value (EUTECH, Instruments), titratable acidity (IS: 1166,1986) of prepared curd samples were determined following standard procedures.

Spontaneous syneresis of undisturbed set curd was determined as per Cartasev and Rudric (2017). The samples were weighed (W1) and kept at an angle (45°) for 20 minutes to allow collection of whey at the sides of the container. Whey from the surface of the sample was siphoned out carefully using a syringe. The curd samples were weighed again (W2). Syneresis was expressed as the per cent weight of the whey over the initial weight of the curd sample.

$$\frac{(W1\text{-}W2) \times 100}{W1} = \text{per cent syneresis}$$

Curd tension was measured using a penetrometer as described by El-Nagar (2013) with a needle probe. The curd samples were kept at 4 ^{0}C for 2 h before taking measurements. The depth of penetration attained in the sample on application of uniform pressure was directly read from the penetrometer. The result was expressed in millimetres as a penetration unit.

From the sample, a small amount was taken and mounted directly on aluminium SEM stubs coated with carbon, without prior fixation, air dried at 40 ^{0}C and sputter coated with gold (Kokkinosa *et al*., 1998). The samples were then observed and photographed with a scanning electron microscope, Tescan (TESCAN VEGA-3-LMU, Czech Republic). Because the method relies on the accuracy of measurements, care was taken to always use the microscope at the same viewing angle.

Statistical analysis

Statistical analysis was done by using the SPSS 21.0 software. Significant differences between treatments were tested by analysis of variance (ANOVA) followed by Duncan posthoc, with levels of significance of $p < 0.05$ and $p < 0.01$.

Results and discussion

The EPS production potential of isolates was evaluated qualitatively by streaking on Congo red agar supplemented with five per cent sucrose. Glucans in EPS react with the Congo red dye giving black colonies whereas non-EPS producing strains form colourless colonies (Freeman *et al*., 1989). According to the results, Pediococcus produced EPS and *L.lactis* did not produce EPS. Moreover, high turbidity when propagated in broth suggested copious EPS production for the indigenous strain. Citrate metabolism plays an important role in fermentations mediated by LAB. Kempler's medium containing potassium ferrocyanide and ferric citrate was used to find citrate-utilising organisms. In the presence of citrate, the reaction between the ferric ions and potassium ferrocyanide is inhibited. Bacteria capable of utilising citrate remove citrate thereby initiating the reaction between these ions which results in the formation of Prussian blue colonies Bacteria unable to utilise citrate appear as white coloured colonies (Kempler and McKay, 1980). According to the observations, *L. lactis* was incapable of utilising citrate. Prussian blue colonies obtained by *P.pentosaceus* indicated its ability to utilise citrate and there by its flavour production potential (Fig. 1). Citrate is a highly oxidised substrate and no reducing equivalents are produced during citrate fermentation. Metabolic end products like diacetyl and acetaldehyde other than lactic acid impart distinct aroma to food products (Hugenholtz, 1993). *P. pentosaceus* used in this study was found to be a promising citrate fermenter (Fig1). Diacetyl production by *P.pentosaceus* has been reported earlier (Irmler *et al*., 2013). The potential to ferment citrate has the advantage of imparting better sensory appeal for the products.

Fig 1: Citrate utilisation: *P. pentosaceus* DMG01- Prussian blue coloured colonies on Kemplers media

Activity and purity of starter cultures are highly essential criteria for assuring the quality of fermented milk products. The activity rating of the starter culture is usually done by assessing the rate of acid production. According to the results of Horrall and Elliker test, the rate of acid production (Table 1) varied significantly between each of them. Only the mixed culture (non-EPS producer-EPS producer) could be graded as "very active". However, products prepared (37°C for 18h) using all three exhibited remarkable acidity ranging from 0.86±0.040 to 1.105±0.047 per cent lactic acid. In spite of using weak acid producer, all culture combinations gave fermented products with titratable acidity above the minimum stipulated standards (0.45% LA) of FSSAI (2022). It can be assumed that the rate of acid production would have increased during the later stages of fermentation. Such differences in acid production during different stages have been reported by Yang *et al.* (2010). In general, the changes observed in pH correlated well with titratable acidity. Such a decrease in pH with a concomitant increase in acidity has been reported by Kamruzzaman *et al*. (2002). In line with the titratable acidity, pH also differed significantly.

Syneresis is the process by which whey is expelled from curd subsequent to formation of coagulum. Syneresis results from shrinkage of gel and this is related to instability of the gel network and loss of the ability to entrap the serum phase (Walstra, 1993). Syneresis value was the highest (1.72 percent) for sample A prepared using non-EPS producing starter. The syneresis value decreased significantly (1.28 percent) when the EPS producer was included in the starter. In the food matrix, EPS imparts desirable rheological changes like

Table 1. Activity of cultures

Rate of Acid production (% LA)			
A	**B**	**C**	F value
0.28±0.008[a]	0.315±0.012[b]	0.36± 0.008[c]	0.000*

(Each value is the average of triplicate observations)
*The mean difference is significant at 0.05 level
Figures bearing the same superscript do not differ significantly

Table 2. Technological properties of the skim milk coagulum samples

	A	**B**	**C**	**F value**
pH	5.06±0.055[a]	4.64±0.065[b]	4.57±0.026[c]	0.00*
Acidity (percent LA)	0.86±0.040[a]	0.9675±0.045[b]	1.105±0.047[c]	0.00*
Syneresis	1.72±0.329[a]	1.55±0.162[b]	1.28±0.085[c]	0.004*
Penetrometer reading (10 ths mm unit)	36.25±1.25[a]	32±0.81[b]	30.75±0.95[c]	0.002*

(Each value is the average of triplicate observations)
*The mean difference is significant at 0.05 levels
Figures bearing the same superscript do not differ significantly.

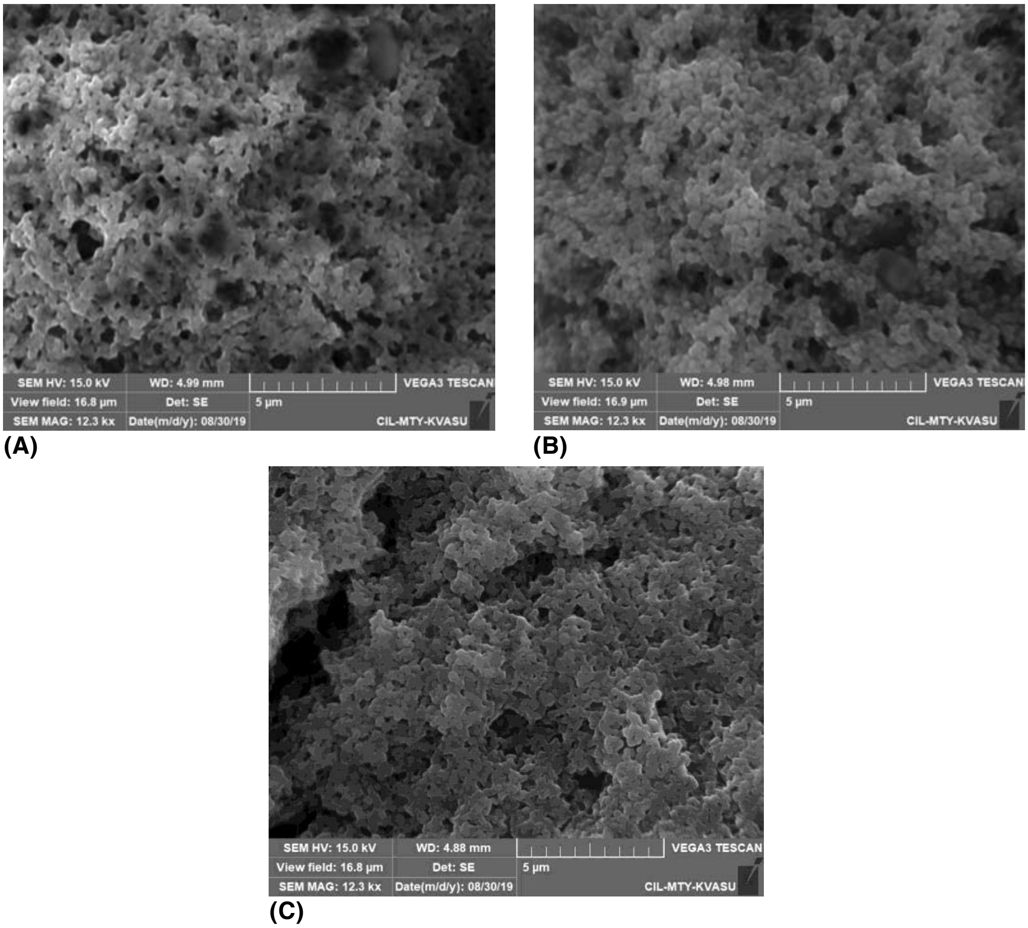

Fig 2: Scanning electron micrograph of skim milk coagulum **A.** Fermentation by *L.lactis* (non-EPS producer) **B.** Fermentation by *P.pentosaceus* (EPS producer) **C**. Fermentation by mixture of *L. lactis and P. pentosaceus* (1:1) In all cases rate of inoculation was two per cent

increased viscosity, improved texture, and reduced syneresis. The observation in this study agrees with Badel *et al*. (2011). The EPS-producing starters confer both rheological and physiological advantages over non-EPS-producing starters (Stack *et al*., 2010). Yang *et al*. (2010) also reported improved water holding capacity and viscosity for yoghurt, when EPS producing *L. rhamnosus* JAAS8 was added to yoghurt starters. Better moisture retention and improved consistency was reported when EPS producing *L. plantarum* was used for cheese preparation (Francois *et al*., 2004). Miao (2015) opined that improvement in texture could be attributed to the polysaccharides which form strands between bacteria and casein micelle. Curd tension is one of the important rheological parameters of fermented milk products. The probe penetrates more when curd tension is low, such that the higher the penetrometer reading the lower the curd tension. In this study highest curd tension was obtained for C, prepared using combination starters. Exopolysaccharide-producing cultures when used as an adjunct starter in yoghurt increased curd tension (Ayana and Ibrahim, 2015). According to Snoeren *et al.* (1976), the electrostatic attraction between molecular casein and charged polysaccharide imparts a stabilising effect. Moreover, rate of acid production also has a decisive role in controlling firmness. Good acid producers yield firmer coagulum. The lowest penetrometer reading with highest acidity observed for sample C endorses earlier reports.

The microstructure of fermented milk has been studied extensively using electron microscopy. Fermented milk coagulum

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possesses a gel-like structure characterised by a network of casein micelle with the liquid phase in the interstitial spaces and the starter bacteria in the void spaces. These arrangements may vary depending on different factors like heat treatment of the mix, type of starter culture, protein content and amount of milk fat and thickening agents (Mahmood *et al.,* 2021). Figure 2 shows the microstructure of samples A, B and C. Distribution of the pores within the protein network differed among samples and were significantly influenced by the type of culture used. The extensive rearrangements of protein particles and EPS produced by the cultures during fermentation lead to the formation of smaller pores and a denser protein matrix. Protein matrix in the sample C is devoid of large interstitial spaces and aggregates making it more compact and thereby less susceptible to syneresis.

Conclusion

The observations in this study indicate the beneficial effect of EPS producing starters on the textural properties of fermented milk product. Microbial exopolysaccharide as bio-thickeners will be helpful in improving texture and mouth feel of low-fat products, which usually have the drawbacks resulting from weak coagulum. The indigenous isolate *P. pentosaceus* DMG01 with its EPS and flavour production potential has to be exploited commercially as an adjunct starter for improving the rheological properties of fermented milk products.

Acknowledgement

The authors thankfully acknowledge Kerala Veterinary and Animal Sciences University for the funding provided for this work.

Conflict of interest

The authors declare that they have no conflict of interest.

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