Molecular characterization of an exopolysaccharide from a probiotic *Lactobacillus plantarum* MTCC 9510 and its efficacy to improve the texture of starchy food

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Abstract The weight average molecular weight (M_w), number average molecular weight (M_n) and size average molecular weight (M_z) of an exopolysaccharide from probiotic *Lactobacillus plantarum* MTCC 9510 was found to be 2.68×10^5 Da, 2.55×10^5 Da and 2.83×10^5 Da, respectively by Gel permeation Chromatography employing the third order polynomial model. The polydispersity index (M_w/M_n) of the polysaccharide was obtained as 1.05. The exopolysaccharide and the starch-exopolysaccharide hydrocolloid exhibited a non-Newtonian and pseudo-plastic behaviour with improvement in the texture of starch containing food by preventing syneresis.

Keywords Probiotics · Exopolysaccharides · Hydrocolloid · Mass spectrometry · Syneresis

Introduction

Exopolysaccharides are long-chain polysaccharides containing branched, repeating units of sugars or sugar derivatives. The major sugar units in exopolysaccharides are glucose, mannose, galactose and rhamnose in different ratio (DeVuyst et al. 2001). The demand for natural polymers for various industrial applications has led to an increased attention towards exopolysaccharides. Exopolysaccharides (EPS) produced by food-grade lactic acid bacteria with GRAS (Generally Recognized as Safe) status are an important source of natural alternatives to commercial additives of plant or animal origin. They have reproducible physico-

chemical properties and are non-toxic with immunostimulatory, antitumour and antioxidant activity (Chabot et al. 2001; Hosono et al. 1997). The physiological function of EPS is one of the most and first biological defence systems against antibiotic, phagocytosis, phage attack and physical stresses which are involved in adhesion and biofilm formation (Looijesteijn et al. 2001; Ruas-Madiedo et al. 2002). EPS from LAB have potential application in improvement of rheology, texture and mouth-feel of fermented products (Welman and Maddox 2003). Their utility is reinforced by the probiotic characteristics of the strain which provide health benefits to the consumers. The nature of EPS produced mainly depends on the strains, culture conditions and medium composition used (Looijesteijn and Hugenholtz 1999). The EPS often differ by monosaccharide composition, linkages between units, presence of repeated side-chains and substitutions.

The structural diversity of EPS among Lactic acid bacteria creates a necessary situation of detailed structural study of the molecule. Most of the experimental data has been obtained by NMR spectroscopy, that enabled the complex equilibrium of inter converting forms of reducing sugars to be unravelled (Angyal 1992). The molecular weights of EPS are also extraordinarily heterogeneous. With incomplete stringent control over the number of subunits added to a chain (Batchelor et al. 1991), long and short polymers are synthesized, although one molecular weight species predominates.

The present work illustrates the molecular weight determination of EPS purified from *Lactobacillus plantarum* MTCC 9510 exhibiting prominent probiotic features as acid, bile and salt tolerance, anti-microbial activity, Gram-positive spectrum antibiotic resistance or sensitivity and mucin binding ability. Size Exclusion Chromatography (SEC) and Matrix-assisted laser desorption ionization-Time of flight (MALDI-TOF) were employed for the determination of EPS molecular weight. Since the primary structure, solution conformation and molecular weight of an

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exopolysaccharide play a role in immune function and performance of the food harbouring the polymer, the present characterization study become extremely important.

Another important aspect discussed here is syneresis in starch containing foods. Syneresis is an important phenomenon observed in food industry. Dairy foods (yogurt, rennet gels, curd, ice-creams, jams etc.) as well as starch based foods (bread, pasta, noodles, soup, salad dressings, jellies, puddings, white sauce, ketchup etc.) are under the stress of syneresis as it affects the functional and sensory properties of the food. This could be controlled by chemical modifications, but blending starch or milk products with polysaccharide hydrocolloids can function as an alternative to the expensive chemical modification (Appelqvist and Debet 1997; Korus et al. 2004). Over the past few decades, interest in using microbial exopolysaccharides in food processing has been increased because of three main reasons: (a) their nature of polydispersity (b) water binding and low solution viscosity (c) their ease of production by fermentation and their ease of manipulation by recombinant DNA technology (Johns and Noor 1991; Morris 1995). However, there has been not much report where the EPS purified from LAB used as an ingredient in food preparations to prevent syneresis. All the effects discussed earlier are based on the in-situ production of exopolysaccharide by LAB cultures present in the food products. The present work is an investigation into the syneresis prevention ability of EPS, purified from *Lactobacillus plantarum* MTCC 9510, in starch stored at cold temperature. Starch/hydrocolloid mixtures are widely used to modify and control the texture of foodstuffs. The addition of a hydrocolloid strongly influences the gelatinization and retro-gradation of starch. It has been demonstrated that the structure of the hydrocolloid, including the type and number of monosaccharide backbone as well as the type, number, and distribution of side units, determines its characteristics and behaviour in solutions. Understanding such properties will lead to improvements in the formulation of starch-based foods. With this point of view, the impact of exopolysaccharides from *Lactobacillus plantarum* on prevention of syneresis in starch and their rheological characterization is examined. The investigation pointed to the fact that the incorporation of the *L. plantarum* EPS powder in a particular concentration showed significant and satisfactory performance in the syneresis prevention as well as the appearance and texture properties of starch.

Materials and methods

Bacterial strain and media

Lactobacillus plantarum MTCC 9510 having vital probiotic features is a facultative anaerobe isolated from curd

(Aswathy et al. 2008). The organism was sub-cultured in de Man–Rogosa–Sharpe (MRS) medium (Himedia, Mumbai) and was maintained in MRS agar at 4 °C for immediate use and prepared 20 % (w/v) glycerol stocks for long time preservation at –80 °C.

Exopolysaccharide production, extraction and purification

18 h old inoculum (10^9 CFU/ml) was prepared in MRS medium by incubating in static condition at 37 °C. EPS production was achieved in EPS production medium (pH 7.3), comprising lactose, yeast extract, ammonium sulphate, di-potassium hydrogen phosphate, sodium acetate, magnesium sulphate, manganese sulphate and tween 80 (Ismail and Nampoothiri 2010a), incubated at static condition at 37 °C for 72 h. The culture was centrifuged at $11,500\times g$ for 15 min at 4 °C to remove cell pellet. Double volume-chilled ethanol used to precipitate the supernatant and incubated overnight at 4 °C. The mixture centrifuged at $2,500\times g$ for 20 min and the pellet collected was dissolved in demineralised water, and again precipitated using double-volume cold ethanol. It was further centrifuged at $2,500\times g$ for 20 min to collect the pellet. The total carbohydrate present in the pellet was estimated by phenol – sulphuric acid method (Dubois et al. 1956). The pellet was dialysed using 5 kDa against distilled water for 24 h with two changes of water and lyophilized. The lyophilized crude exopolysaccharide was purified according to the protocol described by Ismail and Nampoothiri (2010a).

Molecular weight determination of EPS by Size Exclusion Chromatography (SEC) and MALDI-TOF

Molecular weight of the pure EPS was determined by Size Exclusion Chromatography and further confirmed by Matrix-assisted laser desorption ionization (MALDI) mass spectrometry. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry was performed on an Axima CFR⁺ spectrometer (Applied Biosystems, Boston, MA) in the positive and negative modes, using a matrix of 10 mg/ml dithranol in Tetrahydrofuran (THF). Samples were desorbed with a nitrogen laser (λ 330 nm) using a detector sensitivity of 1,000 mV FS. Mass spectra were recorded over an m/z range of 0–200 KDa and represent the summation of 200 acquisitions.

Pure EPS of concentration 10 mg/ml in de-ionized water filtered through 0.22 μ m filter membranes was loaded onto a Sephadex G-200 column (2 $\times$ 30 cm). The column was equilibrated using de-ionized water and the sample eluted using de-ionized water at a flow rate of 1 ml/min using a peristaltic pump. Fractions of 1 ml were collected. Total carbohydrate content of the fractions was determined by phenol-sulphuric acid method (Dubois et al. 1956). Void

volume of the column was determined using blue dextran (Fluka, U.K) at a concentration of 1 mg/ml. Standard dextran (0.3 mg/ml) (Sigma, USA) of molecular weights 150, 50, 22.6, 13.5 and 5 kDa were used to build a calibration curve. The presence of dextran was determined by measuring the spectrum between 280 and 190 nm, which gave a maximum absorbance at 191 nm. The molecular weight of the major eluted fraction was determined from the calibration curve developed using standards by SEC and confirmed by MALDI-TOF. The calibration curve was modelled using a third order polynomial equation to find out the correlation coefficient.

Molecular weight and polydispersity index (PDI) of EPS by High Performance Size Exclusion Chromatography (HPSEC)

Weight average molecular weight (Mw), number average molecular weight (Mn) and size average molecular weight (Mz) of pure exopolysaccharide from *L. plantarum* was determined by GPC. The experiment was carried out in a GPC instrument (Shimadzu, Japan), equipped with GPC data processing software. Phenogel columns 10E6A and 10E3A were connected in series for the experiment. The columns were calibrated with polystyrene standards 271,000, 96,000, 74,500 and 12,500 Da (3 mg/ml). The EPS (3 mg/ml) was eluted using tetrahydrofuran and operated isocratically at a flow rate of 0.6 ml/min. The injection volume was 20 µl.

Exopolysaccharide in prevention of syneresis in starch

The role of *L. plantarum* EPS in the prevention of syneresis in cooked starch pastes during refrigeration was investigated up to 20 days. Starch and polysaccharide dispersions were prepared according to the protocol described by Ismail and Nampoothiri (2010b). Different concentrations of starch (2–6 % w/v) were evaluated and 6 % (w/v) was selected for subsequent studies since the syneresis was more in 6 % wheat starch and it was stable throughout the storage. The effect of increased concentration of EPS in the prevention of syneresis was studied at 0.2–1 % (w/v). The prevention of syneresis was estimated by measuring the liquid phase length (Δh) separated above the sedimented phase in 2 day interval time throughout the storage at 4 °C for 20 days. The efficiency of *L. plantarum* EPS was compared with carboxy methyl cellulose (CMC) as a standard.

Rheological characterization of wheat starch-exopolysaccharide hydrocolloid

The rheological characterization of wheat starch, wheat starch-carboxy methyl cellulose and wheat starch-

exopolysaccharide was studied using Paar Physica Moderate Compact Rheometer MCR 15. Test samples were subjected to shear rate 0–100 s⁻¹ at 25 °C. The apparent viscosity of the samples was calculated at each point and the relationship between shear rate and shear stress was evaluated. The apparent viscosity was expressed in mPa s. The behaviour of wheat starch-exopolysaccharide at low and high concentrations of EPS was evaluated.

Experimental statistics

All experiments have been performed in triplicates and the results represented by their mean $\pm$ SD (standard deviation). The experiments were also statistically analysed employing Analysis of Variance (ANOVA) to see the effect of selected factors on the response. Table 1 shows the details of experiments, factors and their statistical report.

Results and discussion

Molecular weight determination of EPS by Size Exclusion Chromatography (SEC) and MALDI-TOF

Size-exclusion chromatography (SEC) is a chromatographic technique in which analytes are separated based on their molecular size in solution. It is used to determine molecular mass (weight) distribution (MWD) of both natural and synthetic polymers. The void volume of the column and the elution volume of the standards were obtained from graph, plotting absorbance against eluted fractions. The partition coefficient of the standards (M1 150000, M2 50000, M3 22650, M4 13580, M5 5000) and the sample, calculated based on the void volume of the column and their elution volumes, were 0.15, 0.42, 0.54, 0.60, 0.79 and 0.19 respectively. The logarithm of molecular weight of the standard dextrans as a function of partition coefficient was plotted to find out the molecular weight of the polymer. The data was modelled using a third order polynomial equation as it fits to the model. The coefficient of multiple determinations (R^2) was obtained as 1, which indicates the perfect fit of the model developed with higher order polynomial equation. The calibration curve obtained in SEC is not linear and curvilinear data is modelled using higher order polynomial equation. Use of a higher-order polynomial, implies automatically that the model will fit the data better. A major fraction of molecular weight $\sim 1.31 \times 10^5$ Da was obtained from the chromatographic separation, although polysaccharide fractions were obtained in a range of 203–6.5 KDa. The major fraction molecular weight was confirmed by MALDI-TOF, which gave a single peak at 132 KDa very near to the value obtained from the calibration curve, which indicates that the molecular weight obtained by both techniques give

Table 1 Statistical analysis report employing analysis of variance (ANOVA)

Experiment	Source of variation	Sum of squares	df	Mean square	F	P-value	F crit
Exopolysaccharide in prevention of syneresis in wheat starch (6 %)	Day	1.25	10	0.13	64.29	<0.001	2.03
	Percentage of <i>L. plantarum</i> EPS (0.2–1 %)	0.77	5	0.15	79.43	<0.001	2.40
Exopolysaccharide in prevention of syneresis-comparison of <i>L. plantarum</i> EPS with CMC (0.2 %)	Shear rate	92203796	29	3179441	12.77	<0.001	1.86
	Hydrocolloid (<i>L. plantarum</i> EPS& CMC)	1248628	1	1248628	5.02	0.03	4.18
Rheological characterization of wheat starch-exopolysaccharide hydrocolloid	Shear rate	1.7E + 08	29	5867510	214.12	<0.001	1.86
	<i>L. plantarum</i> EPS (0.2 & 1 %)	278311	1	278311	10.16	0.003	4.18

same results. The lower molecular weight fractions could be mainly the oligomers present in the polysaccharide.

Molecular weight and polydispersity index (PDI) of EPS by High Performance Size Exclusion Chromatography (HPSEC)

A calibration curve was plotted with logarithm of molecular weight as a function of retention time with the polystyrene standards. Based on the third order polynomial equation developed for the model, EPS subjected to GPC was found to have a weight average molecular weight (M_w) of 2.68×10^5 Da, number average molecular weight (M_n) of 2.55×10^5 Da and a size average molecular weight (M_z) of 2.83×10^5 Da (Fig. 1). The PDI (M_w/M_n) of the exopolysaccharide was obtained as 1.05, which is a measure of the distribution of molecular mass in the sample. The PDI has a value ≥ 1 . Difference in molecular weight distribution (MWD) pattern was observed in the two gel permeation experiments with the same sample. This difference in the distribution could be attributed to the difference in the standards, mobile phase and detector. The result obtained with the purified EPS produced by *L. plantarum* MTCC 9510 using EPS production medium formulated by the authors coincides with the earlier reports on EPS obtained from lactic acid bacteria. De Vuyst and Degeest (1999) and Vijayendra et al. (2008) reported about the heteropolysaccharides of molecular mass 10^4 to 10^6 Da. Similarly, EPS fractions of molecular weight in the range 3.3×10^4 – 1.32×10^6 Da was also isolated from *Lactobacillus* sp. CFR-2182 (Vijayendra et al. 2009)

Exopolysaccharide in prevention of syneresis in starch

Prevention of syneresis was observed with the incorporation of EPS in all starch concentrations (2–6 % w/v) and EPS concentrations in the range of 0.2–1 % (w/v) incorporated in 6 % (w/v) starch studied since it found to be effective. Even though, inhibition in starch retro-gradation was observed with all concentrations, the rate of inhibition was almost different in all cases. A rate of inhibition of around 50 %

was observed with 1 % EPS while it was only 8 % with 0.2 % EPS (Fig. 2). All other concentrations displayed a percentage of inhibition between these values. The ability of EPS to bind water, promoting the syneresis prevention on addition to cooked starch pastes was proved with the experiment incorporating EPS in starch containing foods. Phase separation in aqueous solutions was observed at higher

Fig. 1 a Gel permeation chromatogram of purified exopolysaccharide b Molar Mass Distribution (MMD) pattern

Fig. 2 Prevention of syneresis in wheat starch (WS) by *Lactobacillus plantarum* exopolysaccharide (EPS) at different concentrations (0.2–1 % (w/v)). Results are the mean value of triplicate experiments

concentrations of *L. plantarum* EPS, thereby bringing out an enhancement in the viscosity of the mixed system. EPS prevent syneresis and improve product stability firstly by increasing the viscosity and elasticity of the final product, and secondly by binding hydration water. Hydrocolloids are mainly used to improve mouth-feel, texture, visual and taste perception, storage stability, mechanical protection and prevention of syneresis in the final food products (Broadbent et al. 2003; Hassan et al. 2003; Sikora et al. 2003). CMC or cellulose gum is presently used in food science as a viscosity modifier or thickener, and to stabilize emulsions in various products including ice-cream. Taking into consideration this aspect, low viscosity CMC was used as a control to compare with EPS from *L. plantarum* which is of low viscous nature at a concentration of 0.2 %. The particular concentration of the hydrocolloids was chosen for comparison as a minimum concentration. Usually, polymers exhibit significant influence in their flow behaviour even from 0.2 % and hence are used at lower concentrations in food preparations in the range of 0.2–1 %. On comparison, the EPS from *L. plantarum* was found to have a better role than CMC in prevention of syneresis in cooked starch pastes. This experiment focuses the disadvantage of CMC in prevention of syneresis as well as the significant performance of EPS from *L. plantarum*. The experiment could contribute into the functional aspect of EPS from *L. plantarum* in food industry by investigating its role in the prevention of syneresis in starchy food stored in cold temperature. The experiments showed that the incorporation of exopolysaccharides at a particular concentration ranging from 0.1 to 1 % in starchy foods can increase the viscosity of the food as well as improve the appearance and texture properties of the food. The incorporation of EPS at a concentration of 1 %

could reduce syneresis by 50 % and increase viscosity by 28 %. The statistical analysis report (Table 1) indicates that the factors chosen for the studies, incubation day and concentration of *L. plantarum* EPS were significant with high impact on the response (degree of syneresis). It was evident from the average value calculation that the degree of syneresis increases with incubation day and decreases with increase in the concentration of *L. plantarum* EPS. The F value higher than critical F value with significant P -value supports the experiment.

The impact of exopolysaccharide in the prevention of syneresis in starch throws light into its use as an ingredient in starch-containing foods for better texture and mouth-feel. The improvement of viscosity of the final product and prevention of syneresis makes the *Lactobacillus plantarum* EPS an effective substitute of CMC as viscosifier in foods.

Mali et al. (2003) described the clear syneresis reduction of cold stored starch pastes after adding guar gum and xanthan gum. Heyman et al. (2010) also observed the reduction of syneresis in commercial béchamel sauce using non-starch hydrocolloids as Mandala et al. (2004) demonstrated a marked effect of xanthan on the syneresis of white sauces.

Rheological characterization of wheat starch-exopolysaccharide hydrocolloid

The rheological behaviour of wheat starch, wheat starch-carboxy methyl cellulose and wheat starch-exopolysaccharide hydrocolloid was investigated by shear stress controlled rheometry. It is speculated that the increased viscosity of EPS-containing foods may increase the residence time of ingested fermented product in the gastrointestinal tract and therefore be beneficial to a transient colonisation by probiotic bacteria. Figure 3 displays the changes in the apparent viscosities of

Fig. 3 Pseudo-plastic behaviour of wheat starch (WS-water) suspension, wheat starch-carboxy methyl cellulose (WS-CMC), wheat starch-exopolysaccharide (WS-EPS) hydrocolloids. Results are the mean value of triplicate experiments

wheat starch-water, wheat starch-carboxy methyl cellulose and wheat starch-exopolysaccharide dispersion. It was found that the starch-EPS hydrocolloid displayed higher apparent viscosity than the one without EPS. The incorporation of the hydrocolloid at a concentration of 0.2 % could increase the viscosity by 14 %. The viscosity of the starch-EPS hydrocolloid was higher than the control, starch-carboxy methyl cellulose hydrocolloid at same concentration. Increasing the concentration of EPS in the starch suspension increased the apparent viscosity of the mixture. ANOVA (Table 1) for the experiment showed that there is significant increase in the viscosity when *L. plantarum* EPS was used instead of CMC. Similarly, significant change in viscosity was noted at lower shear rate on comparison to a higher shear rate. The increase in the concentration of *L. plantarum* EPS was also found satisfactory with significant values. The *F* values obtained were higher than the critical *F* values with highly significant *P*-values. In studies undertaken on starch/hydrocolloid blends, synergistic effects were observed that resulted in an increase in the viscosity of the mixtures compared with starch or hydrocolloid alone (Sandstedt and Abbott 1964).

The dispersions exhibited a non-Newtonian and pseudo-plastic behaviour. The relation between shear stress and shear rate was not linear, which is the characteristic of a non-Newtonian fluid. The dispersions exhibited a shear-thinning behaviour as the apparent viscosity showed an increase at lower shear rate and decrease at higher shear rate. The shear-thinning behaviour of the dispersions is more evident when both apparent viscosity and shear rate are plotted in logarithmic scale (Fig. 4). From the figure, it is evident that the dispersions obey the power-law. Power-law region, i.e., the straight line region, is the portion

Fig. 4 Pseudo-plastic wheat starch-exopolysaccharide (WS-EPS) dispersions obeying power-law

where the apparent viscosity is not at all constant. Similar behaviour was observed for the wheat starch-exopolysaccharide dispersions in all the concentrations of exopolysaccharide.

The particular properties exhibited by the exopolysaccharide could be attributed to the particular structure composition of the EPS comprising glucose and mannose linked by α and β (1, 3) linkages (Ismail and Nampoothiri 2010a) and its molecular weight. The application of exopolysaccharides in varying industrial areas is mainly due to their rheological properties that allow the formation of viscous solutions at low concentrations (0.05–1.0%) and stability over wide temperature, pH and ionic strength ranges (Kumar and Mody 2009). Incorporation of hydrocolloids in dairy foods or beverages is a well-known strategy to provide viscosity, stability and water-holding capacity. This strategy of incorporation of hydrocolloids to provide the above characteristics has been made use of in the present experiment. Pichler et al. (2012) showed that guar gum (hydrocolloid) additions into raspberry cream fillings had a greater impact on the cream filling consistency. Palomba et al. (2012) also described the use of EPS in improving rheological properties in sourdough. It was described by Palomba et al. (2012) that the presence of EPS contributed to the formation of a dough structure with increased viscoelastic characteristics.

The success of EPS application mainly depends on its ability to bind to the water and its efficacy to increase the viscosity of the final product and these were successfully attained in this particular case. This thermal stable EPS from *Lactobacillus plantarum* isolate (Ismail and Nampoothiri 2010a) along with its ability to bind water and increase viscosity, find its role in food industry as it can withstand heat processing and can protect foodstuffs during storage and transportation.

Conclusion

A detailed structural study of exopolysaccharide from *Lactobacillus plantarum* has not been reported till now especially from a probiotic strain, although many other species of *Lactobacillus* has been studied. This is an indigenous isolate showing many probiotic features. The functions of EPS being entirely dependent on the structure and molecular weight and thus the molecular weight determination have become important. The study also revealed that the incorporation of exopolysaccharides in starchy foods can increase the viscosity of the food and thereby improve the appearance and texture properties of the food. The impact of exopolysaccharide in the prevention of syneresis in starch throws light into the use of LAB EPS as an ingredient in starch-containing foods for better texture and mouth-feel.

The improvement of viscosity of the final product and prevention of syneresis makes the *Lactobacillus plantarum* EPS an effective substitute of CMC as viscosifier in foods.

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References

- Angyal SJ (1992) The composition of reducing sugars in solution: current aspects. *Adv Carbohydr Chem Biochem* 49:19–35
- Appelqvist IAM, Debet MRM (1997) Starch-biopolymer interactions—a review. *Food Rev Int* 13:163–224
- Aswathy RG, Ismail B, John RP, Nampoothiri KM (2008) Evaluation of the probiotic characteristics of newly isolated Lactic acid bacteria. *Appl Biochem Biotechnol* 151:244–255
- Batchelor RA, Araguchi GE, Hull RA, Hull S (1991) Regulation by a novel protein of the bimodal distribution of lipopolysaccharide in the outer membrane of *E. coli*. *J Bacteriol* 173:5699–5704
- Broadbent JR, McMahon DJ, Welker DL, Oberg CJ, Moineau S (2003) Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: a review. *J Dairy Sci* 86:407–423
- Chabot S, Yu H-L, Léséleuc LD, Cloutier D, Clasteren M-RV, Lessard M, Roy D, Lacroix M, Oth D (2001) Exopolysaccharides from *Lactobacillus rhamnosus* RW-9595M stimulate TNF, IL-6, IL-12 in human and mouse cultured immunocomponent cells, and IFN- γ in mouse splenocytes. *Le Lait* 81:683–697
- De Vuyst L, Degeest B (1999) Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol Rev* 23:153–177
- DeVuyst L, DeVin F, Vaningelgem F, Degeest B (2001) Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *Int Dairy J* 11:687–707
- Dubois M, Gilles K, Hamilton J, Rebers P, Smith F (1956) Colorimetric methods for determination of sugars and related substances. *Anal Chem* 28:350–356
- Hassan AN, Ipsen R, Janzen T, Qvist KB (2003) Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. *J Dairy Sci* 86:1632–1638
- Heyman B, Depypere F, Delbaere C, Dewettinck K (2010) Effects of non-starch hydrocolloids on the physicochemical properties and stability of a commercial béchamel sauce. *J Food Eng* 99:115–120
- Hosono A, Lee J, Ametani A, Natsume M, Hirayama M, Adachi T, Kaminogawa S (1997) Characterization of a water-soluble polysaccharide fraction with immunopotentiating activity from *Bifidobacterium adolescentis* M101-4. *Biosci Biotechnol Biochem* 61:312–316
- Ismail B, Nampoothiri KM (2010a) Production, purification and structural characterization of an exopolysaccharide produced by a probiotic *Lactobacillus plantarum* MTCC 9510. *Arch Microbiol* 192:1049–1057
- Ismail B, Nampoothiri KM (2010b) Exopolysaccharide production and prevention of syneresis in starch using encapsulated probiotic *Lactobacillus plantarum*. *Food Technol Biotechnol* 48(4):484–489
- Johns MR, Noor E (1991) Recovery and purification of polysaccharides from broth. *Aust J Biotechnol* 5:73–77
- Korus J, Juszczak L, Witczak M, Achremowicz B (2004) Influence of selected hydrocolloids on triticale starch rheological properties. *Int J Food Sci Technol* 39:641–652
- Kumar AS, Mody, K (2009) Microbial exopolysaccharides: variety and potential applications. In *Microbial production of biopolymers and polymer precursors – applications and perspectives*. Caister, Academic Press, Norwich, United Kingdom
- Looijesteijn PJ, Hugenholtz J (1999) Uncoupling of growth and exopolysaccharide production by *Lactobacillus lactis* subsp. *cremoris* NIZO B40 and optimization of its synthesis. *J Biosci Bioeng* 88:78–182
- Looijesteijn PJ, Trapet L, de Vries E, Abee T, Hugenholtz J (2001) Physiological function of exopolysaccharides produced by *Lactococcus lactis*. *Int J Food Microbiol* 64:71–80
- Mali S, Ferrero C, Redigonda V, Grossmann MVE, Zaritzky NE (2003) Influence of pH and hydrocolloids addition on yam (*Dioscorea alata*) starch pastes stability. *LWT – Food Sci Technol* 36:475–481
- Mandala IG, Savvas TP, Kostaropoulos AE (2004) Xanthan and locust bean gum influence on the rheology and structure of a white model-sauce. *J Food Eng* 64:335–342
- Morris VJ (1995) Bacterial polysaccharides. In: Stephens AL (ed) *Food polysaccharides and their application*. Marcel Dekker, New York, pp 341–371
- Palomba S, Cavella S, Torrieri E, Piccolo A, Mazzei P, Blaiotta G, Ventrino V, Pepe O (2012) Polyphasic screening, homopolysaccharide composition, and viscoelastic behavior of wheat sourdough from a *leuconostoc lactis* and *lactobacillus curvatus* exopolysaccharide-producing starter culture. *Appl Environ Microbiol* 78:2737–2747
- Pichler A, Pozderović A, Pavlović J (2012) Influence of sugars, modified starches, and hydrocolloids additions on the rheological properties of raspberry cream filling. *Czech J. Food Sci* 30:227–235
- Ruas-Madiedo P, Tuinier R, Kanning M, Zoon P (2002) Role of exopolysaccharides produced by *Lactococcus lactis* subsp. *cremoris* on the viscosity of fermented milks. *Int Dairy J* 12:689–695
- Sandstedt RM, Abbott RC (1964) A comparison of methods for studying the course of starch gelatinization. *Cereal Sci Today* 9:13–26
- Sikora M, Juszczak L, Sady M, Krawontka J (2003) Use of starch/xanthan gum combinations as thickeners of cocoa syrups. *Nahrung/Food* 47:106–113
- Vijayendra SVN, Palanivel G, Mahadevamma S, Tharanathan RN (2008) Physico-chemical characterization of an exopolysaccharide produced by a non-ropy strain of *Leuconostoc* sp. CFR 2181 isolated from dahi, an Indian traditional lactic fermented milk product. *Carbohydr Poly* 72:300–307
- Vijayendra SVN, Palanivel G, Mahadevamma S, Tharanathan RN (2009) Physico-chemical characterization of a new heteropolysaccharide produced by a native isolate of heterofermentative *Lactobacillus* sp. CFR-2182. *Arch Microbiol* 191:303–310
- Welman AD, Maddox IS (2003) Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends Biotechnol* 21:269–274