THE POTENTIAL OF FRUCTANS PRODUCING ACETIC ACID BACTERIA IN FERMENTED DAIRY PRODUCTS

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Abstract
In this work the combinations of commercial lactic acid bacteria starters and acetic acid bacteria strain were used for production of fructans in substrate, both with and without sucrose additive, and studied their potential in maintaining technological properties of yoghurt and fermented milk. The objective of this study was to assess the effect of fructans producing starter cultures on milk coagulation technique, the amount of secreted fructans and viscosity of fermented milk samples. An amount of fructans synthesized by starter cultures and *Gluconobacter sp.* B35, pH and viscosity of samples were measured using appropriate standards and analytical methods. Results showed that the addition of acetic acid bacteria did not influence the pH dynamics of fermented milk samples. Increasing sucrose concentration in samples significantly influences fructans production potential. The application of such technology in fermented dairy product production would have potential from microbiological exopolysaccharides increasing position with the aim to promote functionality of dairy foods and to substitute commercial stabilisers etc. The addition of acetic acid bacteria in milk showed negative impact on viscosity of the evaluated samples. The viscosity was liquid in all analyzed samples with acetic acid bacteria, the addition of sucrose helped to make the consistency of yoghurt and fermented milk more liquid. We concluded that the structure of synthesized fructans could not help to improve the textural properties of fermented dairy products. From this point of view, the studied acetic acid bacteria strain should have the potential as prebiotic.
Key words: EPS, fructans, lactic acid bacteria, acetic acid bacteria, fermentation.

Introduction

Exopolysaccharides (EPS) producing lactic acid bacteria (LAB), including *Lactobacillus, Leuconostoc, Lactococcus* and *Streptococcus*, synthesize numerous kinds of homopolysaccharides (HoP) and heteropolysaccharides (HeP), diverse in molecular mass, linkages, solubility, and degree of branching (Patel et al., 2010). *Lactococcus lactis* subsp. *cremoris* produce HeP which consists of rhamnose, glucose, galactose and phosphates or EPS that consist of only glucose and galactose (Marshall et al., 1995). *Streptococcus thermpohilus* produce HeP which contained galactose and rhamnose (Vaningelgem et al., 2004b). *Streptococcus thermophilus* strains are known to produce HoP, too. *Leuconostoc spp.* produces dextrans. *Lactobacillus fermentum* produces 100 mg L^{-1} of EPS in MRS (MRS agar with Tween® 80) broth, some strains of species show high apparent viscosity (Kenji et al., 2010). EPS from *L. delbrueckii* contain glucose equivalents and proteins (Canquil et al., 2007).

A lot of work has been done in the field of isolating and characterising the composition of EPS produced by various strains of lactic acid bacteria (Faber et al., 2002; Grobben et al., 2000; Petry et al., 2000; Ruas-Madiedo et al., 2001; Van Calsteren et al., 2002) and other microorganisms. Recently F.Jakob and co-authors (Jakob et al., 2013) identified several strains of acetic acid bacteria (AAB) as being able to produce high amounts of polysaccharides from sucrose. AAB are involved in food biotechnological processes such as vinegar, kombucha or kefir production for their acetic and gluconic acid production (Dufresne, Farnworth, 2000; Giudici and Pullo, 2008; Gulitz et al., 2011). Fructans synthesis is widely spread among bacteria and was also reported for the AAB species *Gluconobacter oxydans*, *Gluconacetobacter xylinus* and *Gluconacetobacter diazotrophicus* (Velazquez-Hernandez et al., 2009).

At present, there is a great potential for the development of systems for heterologous expression and over-production of exopolysaccharides in different food grade bacteria (O'Connor et al., 2007). They could be exploited to increase the EPS content in foods, thereby improving their technological properties and potentially physiological/health characteristics working as prebiotics.

The objective of this study was to assess the effect of fructans producing starter cultures on milk coagulation technique, the amount of secreted fructans and viscosity of fermented milk samples.

Materials and Methods

Materials

Two types of commercial starter cultures Harmony 1.0 and CHN 22 produced by Chr. Hansen (Denmark) were tested. Harmony 1.0 is a part of Yo-Flex cultures (starter culture containing a mixture of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus fermentum)* while CHN 22 is a mixed culture used for production of sour cream, buttermilk, kefir, consisting of a combination of mesophilic strains – *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar diacetylactis, Leuconostoc spp.* (AD Chr. Hansen, 2006). Additionally, *Gluconobacter*

Table 1

The characteristic of analysed samples, their coding

Code	Starter	Sucrose, %
1 (control)	Harmony 1.0	-
1a	Harmony 1.0 + *Gluconobacter sp.* B35	-
1b		4
1c		8
2 (control)	CHN 22	-
2a	CHN 22 + *Gluconobacter sp.* B35	-
2b		4
2c		8

sp. B35 isolated from kombucha tee (Institute of Microbiology and Biotechnology, Latvia University) was added for increasing of fructans in the fermented substrate.

Preparation of samples

Pasteurized and cooled milk samples were inoculated with Harmony 1.0 starter (as control) and with a mixture of Harmony 1.0 and *Gluconobacter sp.* B35 starters; in similar way CHN 22 starter (as control) and a mixture of CHN 22 and *Gluconobacter sp.* B35 starters were used (Table 1). Taking into account the appropriate substrate for fructans production, different sucrose (granulated sugar EU2 550, Nordic Sugar SC, Sweden) concentrations (4 and 8%) were added. Fermentation was led according to the starter producer's recommendations for each culture and stopped when pH reached 4.5. The fermentation was conducted as follows: in case of Harmony 1.0 starter, the fermentation was carried out at 43 °C for 5 h, in case of CHN 22, at 28 °C for 6 h. The amount of starters was added based on the recommendations of starter manufacturer Chr. Hansen (Denmark), but *Gluconobacter sp.* B35 pure culture – 2 mL to 100 g of milk. Culture consists of at least $5*10^7$ CFU g^{-1} acetic acid bacteria.

Fermented milk samples were stirred, cooled and matured at 4-6 °C for 10-12 hours. Samples were analysed immediately after production taking into account EPS degradation possibilities during storage.

Methods

The amount of fructans synthesized by starter cultures and *Gluconobacter sp.* B35, pH and viscosity of samples were measured.

Fructans were determined in fermented milk samples according to fructans assay procedure for the measurement of fructo-oligosaccharides (FOS) and fructans polysaccharide with recombinant inulinases (Megazyme, Ireland) using AOAC Method 999.03 and AACC Method 32.32 procedures.

The pH of samples was measured using pH-meter Jenway 3520.

The apparent viscosity of samples was measured using DV III Ultra Brookfield viscosimeter with the special spindle SC4-16 at a shear rate of $1 min^{-1}$.

Descriptive statistics was carried out to determine the differences of produced fructans concentration in the analysed samples. Correlation analysis was used for determination of the differences between fructans concentration and the apparent viscosity in fermented milk samples.

Results and Discussion

Exopolysaccharides production of LAB is an important attribute for fermented dairy products (Jolly et al., 2002; Welman, Maddox, 2003; Ruas-Madiedo et al., 2010). Our previous experiments (Feldmane et al., 2014) using different Yo-Flex starter cultures (Harmony 1.0, TWIST 1.0 and YF-L902, Chr. Hansen, Denmark) showed that EPS concentration varies from 25.28 to 440.81 mg L^{-1} depending on the fermentation patterns of yoghurt samples. The fermentation temperature significantly contributes to EPS concentration ($p<0.05$) because the increased rate of fermentation is attributed to increased metabolic activity of LAB.

Findings of J.Cerning et al. (1992) and F.Vaningelgem et al. (2004a) showed that optimal temperatures for EPS production were determined as 25 °C for *L. lactis*, 40 °C for *S. thermophilus*, 30 °C for *Leuconostoc spp*. It means that we had chosen appropriate fermentation patterns for fructans synthesis in the present study using lactic acid bacteria starters and lactic acid bacteria starters in the combination with AAB.

For better understanding the influence of fructans producing acetic acid bacteria on the technological properties of fermented milk samples, the pH dynamics of samples during fermentation was studied (see Figure 1 A and B).

The pH dynamics is quite similar during the fermentation of samples. At the end of fermentation, the pH of yoghurt samples ranged from 4.45 to 4.55 and from 4.43 to 4.56 in samples using CHN 22 starter in combination with *Gluconobacter sp.* B35. Results

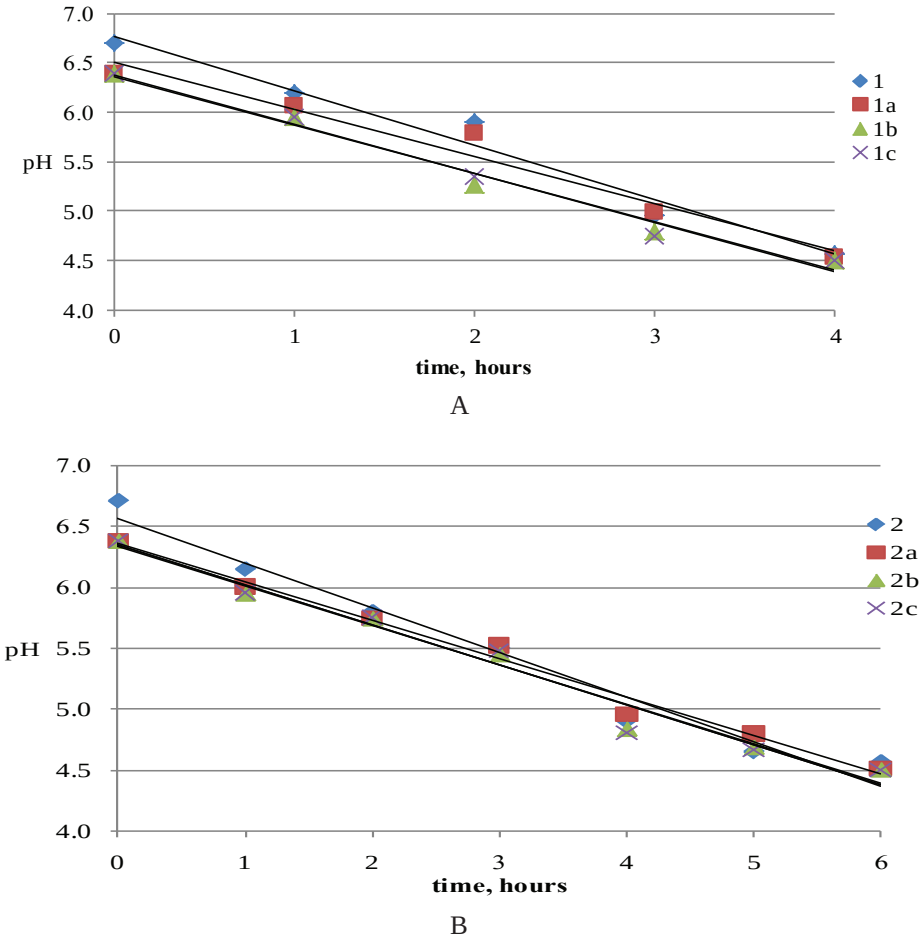

Figure 1. The pH dynamics of samples (A-with Harmony 1.0; B-with CHN 22) during fermentation: 1-Harmony 1.0, 1a-Harmony 1.0+AAB, 1b-Harmony 1.0+AAB+4% sucrose, 1c-Harmony 1.0+AAB+8% sucrose; 2-CHN 22, 2a-CHN 22+AAB, 2b-CHN 22+AAB+4% sucrose, 2c-CHN 22+AAB+8% sucrose.

showed that the addition of acetic acid bacteria had not influenced the pH dynamics of fermented milk samples.

As the aim of present study was to evaluate the potential of acetic acid bacteria on fructans production in milk, we need to clarify that synthesized fructans do not come only from acetic acid bacteria multiplication in fermented substrate. The production of intracellularly synthesized EPS (consisting of glucose and galactose) in yoghurt samples using Harmony 1.0 starter varied roughly from 32.10 to 152.79 mg L^{-1} during the fermentation at temperature interval from 38 to 43 °C (Feldmane et al., 2014), but fructans production potential of experimental samples is summarised in Table 2.

The acquired results show that there is a strong positive linear correlation (r = 0.676) among the amount of added sucrose and fructans content (mg 100 g^{-1}) in fermented milk products. We can declare that the amount of added sucrose increases the fructans production potential in fermented milk products during fermentation.

Table 2

Fructans concentration in analysed fermented milk samples, mg 100 g^{-1}

Sample code	Content of fructans
Harmony 1.0	121
Harmony 1.0+AAB	212
Harmony 1.0+AAB+4% sucrose	241
Harmony 1.0+AAB+8%	256
CHN 22	168
CHN 22+AAB	168
CHN 22+AAB+4% sucrose	184
CHN 22+AAB+8% sucrose	225

Fructans synthesis is catalyzed by fructosyltransferases, which cleave the main substrate sucrose and release glucose in a first step (Jakob et al., 2013). The higher fructans concentrations were observed in 1c, 1b and 2c samples ($p<0.05$). We could explain it with J.Cerning (1990) work conclusions that a mixed culture was characterised with higher

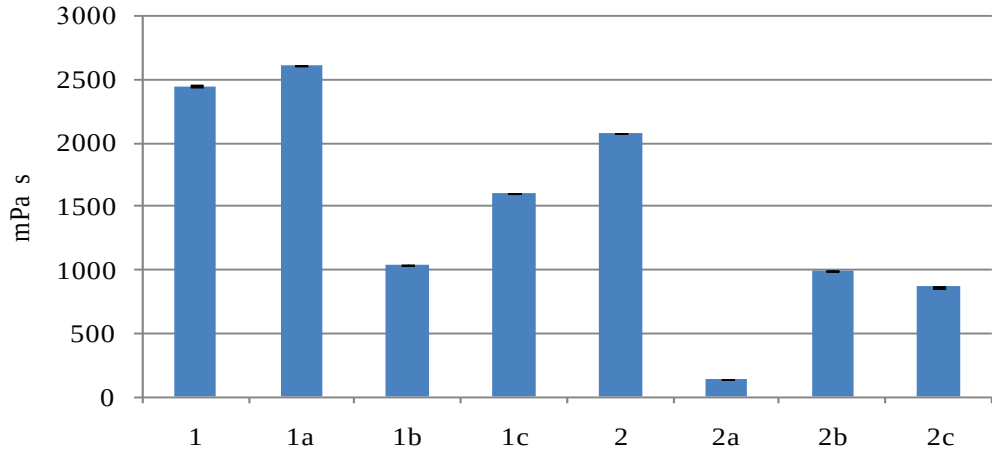

Figure 2. The apparent viscosity of investigated yoghurt and fermented milk samples: 1-Harmony 1.0, 1a-Harmony 1.0+AAB, 1b-Harmony 1.0+AAB+4% sucrose, 1c-Harmony 1.0+AAB+8% sucrose, 2-CHN 22, 2a-CHN 22+AAB, 2b-CHN 22+AAB+4% sucrose, 2c-CHN 22+AAB+8% sucrose.

EPS production potential instead of a single strain. Interestingly, the detected fructans concentrations differed between samples, higher concentrations were observed in yoghurt samples with acetic acid bacteria supplement. These findings were explained by *Streptococcus thermophilus* HeP and HoP production ability and symbioses of starter representatives with AAB during fermentation. Increasing sucrose concentration, significantly influences fructans production potential ($p<0.05$) in yoghurt samples. The application of such technology in fermented dairy products production would have potential from microbiological exopolysaccharides increasing position with the aim to promote functionality of dairy foods and to substitute commercial stabilisers. From negative point of view should be mentioned increased energy value of products.

We know that rheological properties of EPS carry a great importance in their overall characteristics but they are significantly affected by molecular features which need to be understood better (Patel et al., 2010).

The apparent viscosity of samples varied from 131.97 to 2615.58 mPa s (Fig. 2). The highest viscosity was observed in yoghurt samples using Harmony 1.0 starter (1) and Harmony 1.0 starter, and AAB combination (1a). Similar results we found analysing the connection with EPS producing commercial yoghurt starters and viscosity of products (Feldmane, 2013). The presence of *Lactobacillus fermentum* into starter composition helps to increase the apparent viscosity according to information mentioned in the research work of F.Kenji et al. (2010). The 1% (m/V) solution of the purified EPS from *Lactobacillus fermentum* strain showed a high apparent viscosity of 0.88 Pa s at a shear rate of 10 s^{-1}.

We observed a different effect on structural properties of fermented milk samples when acetic acid bacteria and two concentrations (4 and 8%) of the sucrose were added. The addition of acetic acid bacteria in milk showed a negative impact on the viscosity of evaluated samples (1b, 1c, 2a, 2b and 2c). The viscosity was liquid in all investigated samples with AAB with the exception of 1a, also addition of sucrose helps to make more liquid consistency of fermented milk samples. We concluded that the structure of synthesized fructans could not help to improve the textural properties of fermented milk products. Synthesized fructans do not form gels from intermolecular interactions of different polymer chains in milk matrix. We could compare our conclusion with the findings of S. Arvidson et al. (2006) that levan does not form gel from intermolecular interactions of different polymer chains in aqueous solutions and exhibits low intrinsic viscosities even at high molecular weight being typical for spherical particles (Jakob et al., 2013). According to F. Jakob et al. (2013) study, the ability of the isolated levans to bind water should not result from intermolecular interactions of different elongated polymer chains. In fact, intramolecular interactions of individual levan molecules have to be considered to effectively bind water and, therefore, to act as hydrocolloids. The obtained results showed that HeP from LAB play an important role in the rheology of fermented milk products but HoP producers have been evaluated lesser and they are used mainly for fermentation of non-dairy products (Notararigo et al., 2013). From this point of view, the studied fructans of acetic acid bacteria strain should have a higher potential from prebiotic aspect.

Conclusions

This work offers possibilities for further application of AAB (food grade ingredient) strain in dairy products production increasing their functionality and microbiologically synthesized fructans concentration.

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