1.12. MONITORING OF PROBIOTIC AND NON PROBIOTIC LACTOBACILLUS STRAINS' GROWTH BY DIFFERENT PHYSICO-CHEMICAL PARAMETERS

Kaszab T.[1], Szigeti F.[1,3], Bodor Zs.[1], Zinia Zaukuu J-L.[1], Rashed M. S.[1], Slavchev A.[2], Mohácsi-Farkas Cs.[3], Kovacs Z.[1]

[1]Department of Physics and Control, Faculty of Food Science, Szent István University, Hungary
[2]University of Food Technologies, Department of Microbiology, Plovdiv, Bulgaria
[3]Department of Microbiology and Biotechnology, Faculty of Food Science, Szent István University, Hungary
kaszab.timea@etk.szie.hu

Abstract

Lactic acid bacteria (LABs) are Gram+, anaerobic or facultative anaerobic bacteria whose major metabolite end product is lactic acid derived by homolactic fermentation. The aim of our study was to describe the behavior of the different bacteria strains during the evolvement of the yoghurt by various microbiological, physical and chemical methods. In this study, 15 different bacteria strains (genus *Lactobacillus*) were analyzed, which had different probiotic properties. Fatty milk powder was diluted until reaching the protein content with sterilized distilled water and inoculated with the freeze-dried bacteria (starter incubation). The reconstituted milk product was inoculated with the active bacteria culture and the formation of the yoghurt character was monitored for 20 hours at 37°C by rotational viscosimetry, pH measurement. The cell count was determined microscopically after the starter incubation and at the end of the yoghurt cultivation. Results showed that the properties of various strains are different.

Keywords: yoghurt, Lactobacillus, viscosity

Introduction

Lactic acid bacteria (LABs) are non-pathogenic, Gram+ bacteria with anaerob of facultative anaerob metabolism. They require specific nutritional environment and the end product of their metabolism is lactic acid (Slavchev et al., 2015). LABs are commonly associated with the human gastrointestinal tract (GIT), due to their beneficial effect on digestion and physical condition (Remagnia et al., 2013). The industrial importance of LABs is given by their fermentation activity and nutritionally advantage originating from their probiotic activity. Probiotics have numerous beneficial attributes in health such as supporting the immune system, decreasing risk of colon cancer, decrease the symptoms of lactose intolerance and relieve irritable bowel syndrome. The most important property of probiotics is that they can survive in the upper gastrointestinal tract despite the low pH (Slavchev et al., 2017). The demand for probiotic containing food has increased

recently (Michael et al., 2015). Yoghurt is known as the most popular carrier for probiotic bacteria (Fazilah et al., 2018).
Yoghurt is produced from fresh whole or skimmed milk which, is inoculated with bacterial starter culture after pasteurization. This starter culture often contains *Streptococcus salivarus subspp. thermophilus and Lactobacillus delbrueckii subspp. bulgaricus* (Falade et al., 2015). In genus Lactobacillus the resistance for effect of upper GIT is diverse. There are probiotic, moderate and non-probiotic strains, therefore the probiotic activity of youghurt mainly depends on the strain given to the yoghurt (Slavchev et al., 2017). Besides other factors like fermentation process, type of milk and storage conditions, the formation of yoghurt structure is also, influenced by the type of bacterial strain,. These factors also has an effect on the reproduction of bacteria (Fazilah et al., 2018).
The aim of our study was to describe the behavior of the different bacteria strains during the evolvement of the yoghurt by various microbiological, physical and chemical methods.

Materials and methods

Yoghurt making process

In our study 15 different types of Lactobacillus strains of bacteria was used for the production of yoghurt. These strains can be divided into three groups due to their probiotic activity: non-probiotic (N), moderately probiotic (M) and probiotic (P). Fatty milk powder was diluted until reaching the protein content with sterilized distilled water and inoculated with the freeze-dried bacteria (starter incubation). This strain suspension was cultivated for 20 hours at 37°C. The reconstituted milk product was inoculated with the activated bacteria and the following measurements were performed during the formation of the yoghurt process through 20 hours at 37 °C. The whole process was repeated two times for each bacteria and resulted in two yoghurt products from each bacterial strain.

Microbiological cell count

Numbers of cells were counted after the activation of bacteria and also at the end of the formation of yoghurt by Breed Staining Method with light microscope at 100 times magnitude with immersion oil.

Measurement of pH

The pH measurement of forming yoghurt was detected with Mettler Toledo Seven Multi pH meter (Mettler Toledo, USA), in every four minutes through 20 hours and resulted in 300 pH values for each yoghurt product. The pH meter was calibrated between pH 4 and pH 7. The pH values were plotted and the following parameters were determined from each curve: minimum and maximum value, inflexion point. The measuring time of each determined parameter was marked as well.

Rheological measurement

Haake RotoVisco1 (Thermo Scientific, Karsruhe, Germany) rotational viscometer was applied to determine the shear stress of the reconstituted milk product samples with activated bacteria. During the formation of the yoghurt process each sample was measured through 20 hours at 37°C. Each measurement includes four stages. The stages were as the follows: accelerated shear velocity up to 100 1/s during 100s; mixing at the maximum velocity during 100s; slowing velocity from the maximum to zero during 100s, mixing at mminimum velocity (1 1/s) during 3300s. The dynamical viscosity was calculated from the ratio of shear stress and shear velocity. Several parameters were determined from slow and fast stage of the viscosity curves. These paramteres were as the follows: first and second peak, maximum

and last values of the viscosity. The measuring time of each determined parameter was marked as well.

Optical density measurement

Optical density measurement was performed with Ocean Optics ULTRA 2000+ LS1 spectrometer. The spectra were recorded between 600-900 nm in every minute through 20 hours at 37°C. Curves were drawn with respect to time and characteristic periods were determined.

Statistical evaluation of data

ANOVA was used to identify if there is any significant differences between the different groups based on probiotic activity (non-probiotic, moderately probiotic and probiotic) in the case of pH, time and viscosity characteristic parameters. Where ANOVA indicated significant difference, TukeyHSD post-hoc test ($p<0.05$) was used for detecting the significant differences between the groups.

Multivariate statistical methods were applied to evaluate the result of the pH and viscosity measurements. Principal component analysis (PCA) (Cowe & McNicol, 1985) was used to describe the multidimensional patterns of the measured and determined pH, time and viscosity parameters.

Quantitative models were built using partial least squares regression (PLSR) (Naes et al., 2002) for the prediction of the start and final cell numbers using the data of pH, RotoVisco1.

The quantitative models were validated using Leave-one sample-out cross-validation and the models were evaluated by comparing the determination coefficients (R2) and root mean square errors (RMSE) of calibration and cross-validation.

The statistical analysis was performed with R-Studio (v. R-Studio 1.1.414, 2018.).

Results

Results of the pH and rheological measurements

The growth rate was calculated from the ratio of the final and start cell number. Figure 1 shows the growth rate of different bacteria strains in experiment 1 and 2. Difference was found based on the probiotic degree. Furthermore, several differences were found between the various bacteria strain within each type as well.

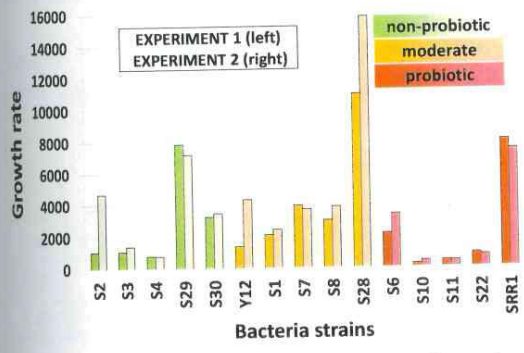

Figure 1. Growth rate of the different bacteria strains

Figure 2 shows the curves of the pH as a factor of time in experiment 1.

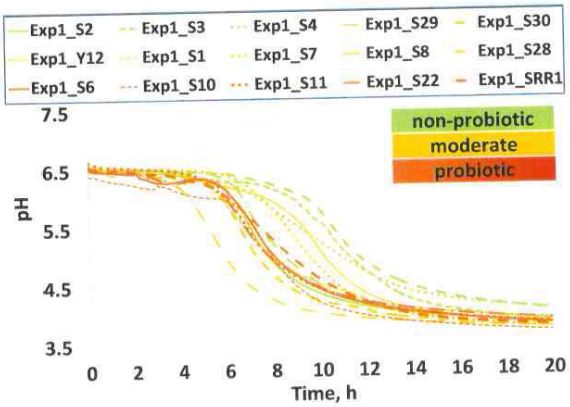

Figure 2. The pH change during of the different bacteria strains in experiment 1

The initial pH of each bacteria strain was very similar and the final pH showed higher standard deviation. The pH curves were diverse according to the different strains. Despite this variability each curve could be divided into three sections, a slower decrease,

followed by a fast decrease, and again a slower decrease section resulting in a saturation curve. Difference was found between the time of non-probiotic and probiotic strains at same pH value.
Inflexion point was calculated from each pH curve, and the time point of inflexion point was determined for experiment 1 (Figure 3). Mean was calculated from the pH value of the samples by the different probiotic groups. Decrease of pH inflexion point was found in contrast to the increase in probiotics ability.

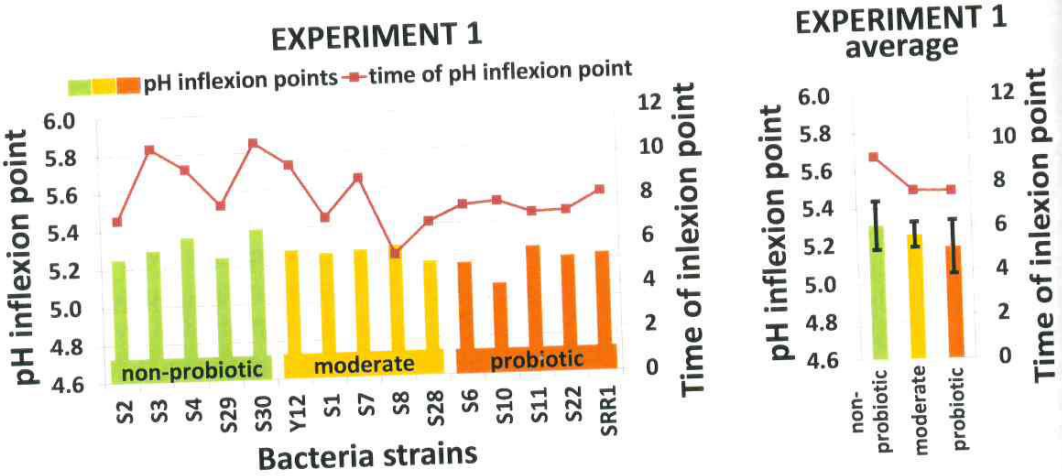

Figure 3. The pH and time in the inflexion points of pH in experiment 1 (left) and average of the experiment 1 (right)

Several specific points were determined on the recorded viscosity curves. These were the follows: 1st peak, maximum value and the final viscosity at 19h in results of slow and fast periods also, furthermore the 2nd peak in the slow periods. The Figure 4 shows the sample S4 from experiment 1.
Each bacteria strain showed different viscosity values at the selected points, however weak similarity was found between the same probiotics samples.
The Figure 5 shows the most specific parameters of the viscosity curves of experiment 1. Difference was found between the non-probiotic, moderate and probiotic ability groups in the determined characteristic parameters despite the variability detected within groups.

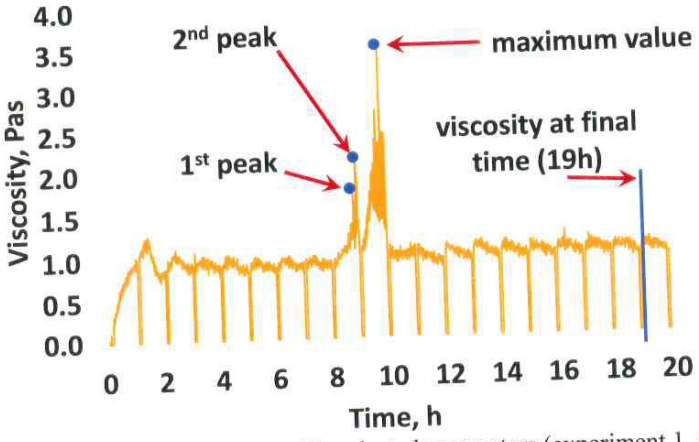

Figure 4. Viscosity curve with the specific selected parameters (experiment 1, sample S4)

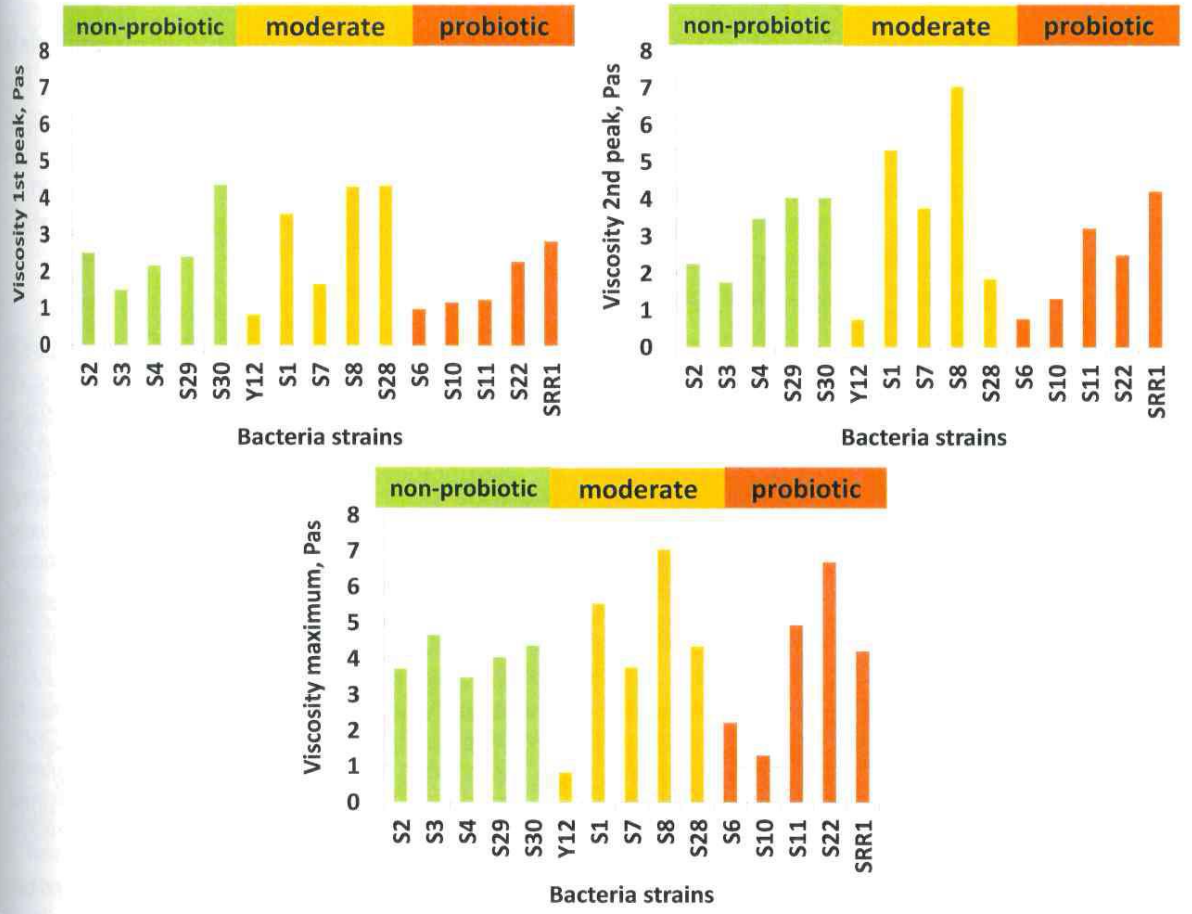

Figure 5. The main selected viscosity parameters (1st, 2nd peak and maximum value) of the different bacteria strains at experiment 1

Results of ANOVA and TukeyHSD test of the determined characteristic parameters

ANOVA and TukeyHSD test showed significant difference between the non-probiotic and probiotic bacteria strains in case of time slow peak 1, time slow peak 2, pH slow max, time fast first peak, pH inf.

Table 1. TukeyHSD test results of the successful selected parameters at experiment 1 ($p < 0.05$)

p<0.05	time slow 1st peak	time slow 2nd peak	pH slow max	time fast 1st peak	pH inflexion point
N-M	< 0.05	< 0.05	< 0.1	< 0.01	-
M-P	-	-	-	-	-
N-P	< 0.05	< 0.05	-	-	< 0.05

Results of the optical density measurement

Similarly, to results of pH the curve of optical density measurement was diverse according to the strain of bacteria. Despite this variability characteristic time periods were found in each curve: the first period was around two hours, the second around 4-5 hours, and the third at around 8-10 hours. Besides, after ten hours a decrease or an increase was observed in optical densityin the different strains. The chosen time periods can be connected to pH curves also, the exact relationship needs further analysis between these parameters.

Discussion

Different bacteria strains showed variable behavior properties in each measured parameter. According to the probiotic activity some parameters showed significant difference mainly in time parameters. The pH and optical density curves showed similar properties for the individual strains therefore these two parameters could have some connection with each other, further analysis is needed to find the exact relationship. The variability of the strains shows that there is an industrial meaning of which strain is used for the yoghurt production, so our result can be useful to find the most appropriate bacteria.

References

Cowe, I. A., & McNicol, J. W. (1985). The use of principal components in the analysis of near-infrared spectra. Applied Spectroscopy, 39(2), 257–266.

Falade, K. O., Ogundele, O. M., Ogunshe, A. O., Fayemi, O. E., & Ocloo, F. C. K. (2015). Physico-chemical, sensory and microbiological characteristics of plain yoghurt from bambara groundnut (Vigna subterranea) and soybeans (Glycine max). *Journal of Food Science and Technology*, *52*(9), 5858–5865.

Fazilah, N. F., Ariff, A. B., Khayat, M. E., Rios-Solis, L., & Halim, M. (2018). Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. *Journal of Functional Foods*, *48*(April), 387–399.

Michael, M., Phebus, R. K., & Schmidt, K. A. (2015). Plant extract enhances the viability of Lactobacillus delbrueckii subsp. bulgaricus and Lactobacillus acidophilus in probiotic nonfat yogurt. *Food Science & Nutrition*, *3*(1), 48–55.

Naes, T., Isaksson, T., Fearn, T., & Davies, T. (2002). A user friendly guide to multivariate calibration and classification. Chichester, UK: NIR Publications

Remagnia, M. C., Moritab, H., Koshibab, H., P., T. M., Cattaneoc, & Tsenkovab, and R. (2013). Evaluation of Digestion Resistance of Lactic Acid Bacteria Using Near-infrared Spectoscopy and Aquaphotomics. In *NIR 2013 - 16th International Conference on Near Infrared Spectroscopy* (pp. 602–608). La Grande-Motte.

Slavchev, A., Kovacs, Z., Koshiba, H., Bazar, G., Pollner, B., Krastanov, A., & Tsenkova, R. (2017). Monitoring of water spectral patterns of lactobacilli development as a tool for rapid selection of probiotic candidates. *Journal of Near Infrared Spectroscopy*, *25*(6), 423–431.

Slavchev, A., Kovacs, Z., Koshiba, H., Nagai, A., Bázár, G., Krastanov, A., Tsenkova, R. (2015). Monitoring of Water Spectral Pattern Reveals Differences in Probiotics Growth When Used for Rapid Bacteria Selection. *PLOS ONE*, *10*(7), e0130698.

Acknowledgement

We are owing thank for Alexander Slachev for providing us the bacteria strains. This paper was supported by the *Bolyai János* Research Sholarship of the Hungarian Academy of Sciences (Zoltan Kovacs).