CORVINUS
UNIVERSITY of
BUDAPEST

Faculty of Food Science

THESES OF THE DOCTORAL DISSERTATION

SCIENTIFIC AND EXPERIMENTAL BASES OF THE PRODUCTION OF NON-DAIRY PROBIOTIC FOODS

SZILÁRD KUN

BUDAPEST
2008

PhD School/Program

Name: PhD School of Food Science

Field: Food Science

Head: Prof. Péter Fodor DSc.
Corvinus University of Budapest

Supervisor: Judit Rezessy-Szabó PhD
Prof. Ágoston Hoschke CSc.
Department of Brewing and Distilling
Faculty of Food Science
Corvinus University of Budapest

The applicant met the requirements of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

Signature of Head of School

Signatures of Supervisors

BACKGROUND AND AIMS OF THE WORK

The concept of nutrition derived a changed meaning as a result of economic and social development. Nowadays nutrition means not merely that essential nutrients and energy is provided for our body, but that on a buyer's market we can choose among foods on the basis of their appearance, pleasure and nourishing value. Since the end of the 20th century main goal of nutrition in the developed countries is to provide balanced nutritional intake for the metabolism of the human body to prevent deficiency or excess of certain components, and guarantee well-being for the consumers. Certain social strata, for example the younger generation and the intellectuals take better care of the nutritional value of the food, and the claim of health-conscious nutrition is borning.

Development of analytical methods makes possible the determination of the exact chemical composition of foods, and the evaluation of the physiological effect of single components. The idea of functional food was proceeded from the above mentioned conception. Functional food possess such characteristics beside nourishing value that have proven positive physiological effect on the human body

Research, design and establishment of functional foods into the food processing are one of the most dynamically growing fields. In order to distinguish them within the category of everyday foods we need scientific arguments that prove the health promoting physiological effects. These evidences make it possible to take health claims on the label of foods that inform consumers about health benefits. Probiotic dairy products are among functional foods consumed for the longest time, and they still dominate the market of probiotics.

Foods cannot be endangering on the health of the consumer. Food safety should be extended to consumers who suffer from food related disorders. Probiotic products have to be made available for them, so they do not have to abandon benefits of probiotics. The functional food of the future should be tailored to the individual, and satisfy the genetic and biochemical needs of the individual. Such genetic disorder may be the sensitivity to milk in form of lactose intolerance or milk protein allergy. This group of consumers cannot eat milk or dairy products or only to a limited extent.

Governed by the above mentioned motivation I have chosen the development of the technology of non-dairy probiotic products as theme of my doctoral research. I have selected different vegetable raw materials to elaborate the technology. The topics of my research were defined on the basis of the scientific and practical problems related to development of the relevant technology.

AIMS OF THE RESEARCH

- Investigation of the physiological characteristics of *Bifidobacterium* strains
  - Examination of vancomycin and hydrogen peroxide sensitivity
  - Classification according to oxygen tolerance
  - Determination of carbohydrate utilization
  - Evaluation of the effects of Maillard-reaction products
- Demonstration of antimicrobial activity of *Bifidobacterium* strains and determination of metabolites taking part
- Modeling of the adherence of *Bifidobacterium* strains *in vitro* to intestinal epithelium applying human tissue culture.
- Determination of the fermentability of different juices made of vegetable raw material with *Bifidobacterium* strains.
  - Comparative evaluation of pretreatment methods for the decrease of initial microbial population
  - Optimization of the parameters of lacto-fermentation to achieve maximal yield of biomass
  - Elaboration of fermentation technologies for the production of vegetable based probiotic products
- Investigation of mixed culture fermentations with *Bifidobacterium* and lactic acid bacterium strains
  - Elaboration of the fermentation technology and scaling-up
- Formulation of fermented product and determination of the survival the probiotic strain during storage in different environment

MATERIALS AND METHODS

I have used 29 *Bifidobacterium*, 8 *Lactobacillus* and 7 potentially pathogenic strains. The *Bifidobacterium* strains originated from the culture collection of the Department of Brewing and Distilling, Corvinus University of Budapest, and from Chr. Hansen. The *Lactobacillus* strains were provided by the Biology Department of the Central Food Research Institute, while the potentially pathogenic strains from the Department of Microbiology and Biotechnology of CUB. The Caco-2, HT29 and HT29-12 cell lines were made available by the Physiology Department of the Szent István University, Budapest. In the prebiotic utilization test I have used fructo-oligosaccharides (Raftiline, Raftilose), lactosucrose, isomalto-oligosaccharides (OligoTime, Oligo MT500 and Isomalto-500), and xylo-oligosaccharides (Xylo-oligo 70, Xylo-oligo 95P). I have used cacao and bread melanoidins to study the effect of Maillard-reaction products that were kindly provided by the Department of Nutrition, University of Vienna. Fermentation experiments were conducted in carrot, beet and Jerusalem artichoke juices.

Methods used for the physiological studies of bifidobacteria

Agar diffusion and limit dilution analyses were used to determine sensitivity for vancomycin and inhibitory concentration of hydrogen peroxide, and to study the effect of melanoidins on the growth of *Bifidobacterium* strains. Oxygen tolerance was examined with cultivation method, in which cells in the late logarithmic growth phase had been incubated under aerobic conditions for 96 hours, and change of the number of living cells was followed. In the investigation of carbohydrate utilization of bifidobacteria optical density and the plate count method were used.

Methods used for the investigation of antimicrobial effect of Bifidobacteria

In order to demonstrate antimicrobial effect of bifidobacteria spot method and the agar diffusion assay were applied. In the case of the spot method I transferred bifidobacterium suspension spots on the surface of the nutrient agar and following 18-hour incubation it was covered with a second layer of agar that was inoculated with the test strain. After a second incubation evaluation was made based on the clear-up zones. Protein profile and molecular mass of the antimicrobial substance were obtained by SDS-PAGE using the following parameters: 15-20% gradient gel and 6% stacking gel, 200 V and 500 mA (at start), 200 V and 14 mA (at the end), 30 minutes running time. Inhibitory effect of protein species of cell-free culture liquids were tested with blotting: after running the gel was placed between two agar layers containing the test strain. In case of the *B. longum* A4.8 the 20 individual protein fractions were obtained with Rotofor IEF Cell apparatus, which separates them on base of

their isoelectric points. Inhibitory effect of the individual protein fraction was tested with the agar diffusion method.

Methods of studying adherence

The different cell lines were cultivated in 24-well plates: the Caco-2 for 14 days, the HT 29 and HT 29-12 cell lines for 72 hours. Cell cultures were washed and the bacterium suspension in the required concentration was transferred onto their surface, and this procedure was followed by 60-minute incubation at 37°C in 5% CO_2 atmosphere. According to the detection method adhering bacterial cell were either removed or fixated to the cell culture. Different methods were used to determine the quantity of the adhering bacteria: plating on various medium, Gram staining (GRAM-color Staining Set, Merck), and hexidium iodide staining (LIVE BacLight^{TM} Bacterial Gram Stain Kit).

Fermentation experiments

Pretreatment of vegetable juices was accomplished by heat treatment and high hydrostatic pressure treatment. Both the mono and the mixed culture fermentations were started with 10^6-10^7 cfu/ml cell number. In case of mixed culture fermentation ratio of bifidobacterium to lactobacillus was 1 to 1. Fermentations were carried out at 37°C in anaerobic environment in anaerobic chamber (Bugbox, Ruskin Technology) or in anaerobic Jar+GasPak system (Oxoid). Scaling-up experiments were accomplished in a Biostat B (BBraun) with 2 liter working volume bench-scale fermentor, in which anaerobic conditions were provided by circulating carbon dioxide and nitrogen gas mixture (10% CO_2 and 90% N_2). Different pectins (Herbstreith & Fox Kft.) – apple pectin (Pectin Classic AJ 201, AJ202, AF703, AU202), citrus pectins (Pectin Classic CJ201, CJ206), and sodium alginate (Satalgine 550, KUK Hungaria Kft.) – were used to create gel-like texture and to decrease water activity of products made with mixed culture fermentation of Jerusalem artichoke juice. Storage experiments were carried out at 4°C or at room temperature in well closed jars to define the survival of probiotics in the various preparations.

Microbiological examinations

Different laboratory and selective media were used to determine concentration of the individual groups of microorganisms: *Bifidobacteria* – TPY and Beerns' agar; lactic acid bacteria – MRS agar; total aerobic mesophilic count – TGE agar; Sulphite-reducing bacteria – Iron-sulphite agar; Enterobacteria – VRBD agar, *Coliform* bacteria – Faecal coliform agar; *Pseudomonas* – Pseudomonas selective agar; Fungi and moulds – RBC agar.

Analytical methods

Concentration of carbohydrates, organic acids and carotenoids were determined by HPLC techniques.

SUMMARY

In order to accomplish the product development that was aimed in my research aim, a strain selection was performed. Within the frame of this I had defined the physiological properties of the relevant *Bifidobacterium* strains, their antimicrobial activity and adherence to the intestinal epithelial cells. Furthermore, I had investigated the applicability of pre-treatment technologies; I had studied the fermenting and growth ability of the strains in different vegetable juices, and the possibility of elaborating a fermentation technology with mono and mixed cultures. Finally, I aimed to carry out scale-up and storage trials.

Physiological study of bifidobacteria

As the primary selection criterion I had surveyed the vancomycin resistance of the probiotic bifidobacteria. Inhibitory effect of vancomycin is of great importance, because this is one of those broad-spectrum antibiotics that have effect against pathogens responsible for clinical infections. All tested strains proved to be sensitive to vancomycin based on my results. I had detected 18 to 44 mm clear-up zones by the agar diffusion method, and 0.75 to 3.0 μg/ml minimal inhibitory (MIC) concentrations in liquid culture.

Since bifidobacteria are catalase negative microorganisms, it is important to get information on their hydrogen peroxide tolerance which may serve as selection criterion when strains are used in mixed culture. The strains *B. lactis* Bb-12 and *B. bifidum* B7.1 proved to be the most resistant ones: in liquid culture 200 μg/ml concentration was defined as minimal inhibitory concentration. The *Bifidobacterium longum* B2.2 strain was the most sensitive with 100 μg/ml MIC value. These results are promising from the point of view of mixed culture fermentations with lactic acid bacteria and bifidobacteria.

I have examined the oxygen tolerance of *Bifidobacterium* strains, which will emerge as important factor in the elaboration of a fermentation technology and in the scaling-up. The strains *B. breve*^{T}, *B. lactis* Bb-12, *B. dentium* B2.1, *B. bifidum* B7.1, *B. longum* A4.4, A4.8 and *B. bifidum* B1.2 were exposed to oxygen in the late logarithmic growth phase for 96-hour period.. Cell counts remained within one order of magnitude for 24 hours, but viability of the tested strains decreased significantly after 96 hours.

Knowledge of the carbohydrate (mono-, di-, oligo- and polysaccharides) utilization of the strains provides help in choosing the right medium (raw material). Furthermore, it is necessary to investigate the extent of the prebiotic effect of oligo- and polysaccharides. The tested strains utilized glucose, lactose, sucrose and maltose generally well as growth substrate, while fructose to a lesser extent. Of the examined *Bifidobacterium* strains *B. adolescentis* and *B. lactis* Bb-12 utilized better all the tested prebiotics than glucose. When studying potentially pathogen bacteria I noticed that they utilize less efficiently Raftiline and the xilo-oligosaccharides than glucose. Based on these results it can be stated that utilization of

prebiotics show variation within species in both bifidobacteria and other bacterium strains, and should be considered as a strain-dependent characteristics. I had successfully established the basis of a mixed culture model system in which the prebiotic effect of commercially available oligo- and polysaccharides can be evaluated.
During pretreatment processes Maillard-reaction products may evolve, thus effect of them on the *Bifidobacterium* strains were explored. From my results I had concluded that melanoidins do inhibit the growth of bifidobacteria to a certain extent. The inhibition depends on the molecular mass of the melanoidins and on the strain as well.

Antimicrobial activity of bifidobacteria

I have investigated the antimicrobial activity of the strains and their proteinaceous inhibitory substance production which may have role in the biological preservation of foods.
Results of the spot method showed that several *B. longum* and *B. bifidum* strains exerted strong inhibition on the growth of the *Escherichia coli* ATCC 8439, *Escherichia coli* O157:H7, *Enterococcus faecalis* and the *Listeria monocytogenes* 4ab strains. Furthermore, I found out that only the *B. longum* A4.8 strain showed antagonistic effect on *Lactobacillus* strains – *Lb. acidophilus* La-5 and *Lb. casei* subsp. *casei* 2756 – while the *B. bifidum* B5.1 inhibited the growth of only the *Lb. acidophilus* La-5 strain. This result is favourable from the point of view of the design of multi-component starter cultures. My results had highlighted that antimicrobial substance production of the strains depend on the growth conditions. Data obtained from the blot method had also confirmed that the supernatant of some *Bifidobacterium* strains contain proteins with antimicrobial activity. The one (No. 2) of twenty fractions obtained by the separation of protein with Rotofor produced by the *B. longum* A4.8 strain had inhibited the growth of all but one test strains – *Enterobacter cloacae*. This clearly indicates the presence of proteinaceous antimicrobial substance.

Adherence of bifidobacteria to human tissue cultures

The bacteria have to adhere to the intestinal epithelium for the development of an interaction and communication between the human body and the bacterial cell. Taking this thought as a starting-point I investigated the adherence of *Bifidobacterium* strains to Caco-2 cell line, and the strains *B. lactis* Bb-12, *B. bifidum* B3.2 and B7.1, and *B. longum* A4.9 showed the best ability to adhere. The number of the adhering bacterial cell increase in direct ratio to the initial cell concentration, but the percentage of adhering bacteria is in inverse ratio to the initial cell count. Based on the results of competitive adherence the *E. coli* Bay100 did not affect the adherence of *B. bifidum* B3.2 or the *B. lactis* Bb-12 strains. Of the tested *Bifidobacterium* strains *B. bifidum* B3.2 did not inhibit the adherence of *E. coli* Bay100. The *B. lactis* Bb-12 showed weak inhibition at 10^8 and 10^7 cfu/well added *E. coli* concentrations, while at 10^6 cfu/well added *E. coli* concentration the extent of the inhibition was one order of a magnitude compared to sole adherence of the *E. coli* strain.

Fermentation experiments

In the course of my fermentation experiment it turned out that the applied mono and mixed cultured utilized well the vegetable juices (carrot, beet and Jerusalem artichoke juice). Beside the pasteurization I have investigated the applicability of high hydrostatic pressure as pre-treatment of the raw carrot juice. I have made a survey of the growth and metabolic activity of bifidobacteria in carrot juice. Specific cell yield in pasteurized carrot juice was the following in the case of the strains: *B. lactis* Bb-12: $2.48*10^{10}$ cfu/l*h, *B. bifidum* B7.1: $6.26*10^{10}$ cfu/l*h, *B. bifidum* B3.2: $7.60*10^{10}$ cfu/l*h, and *B. longum* A4.8 $4.50*10^{10}$ cfu/l*h. These values are in accordance with data found in the literature. Cell count of all four strains reached the 10^8 order of magnitude in the 6^{th} hour of the fermentation. The concentration of carbohydrates decreased gradually in all cases. The rate of carbohydrate decrease was similar in case of the Bb-12, B3.2 and B7.1 strains (from 4.36 (w/v)% to 3.80-3.88 (w/v)%). In case of the strain A4.8 the decrease was smaller, from 4.36 (w/v)% to 4.04 (w/v)%. At the end of the fermentations the lactic acid and the acetic acid concentration was 15-17 mg/ml and 3.3-3.5 mg/ml, respectively. The α- and β-carotene concentration showed different decrease after 24 hours of fermentation: the smallest change was in case of the B7.1 strain (4 and 9%, respectively), while the biggest was in case of the A4.8 strain (22 and 30%, respectively). Based on the experimental results that were aimed at product development it can be concluded that both in carrot juice and in Jerusalem artichoke juice the scaling-up and storage goals were achieved. I had determined the optimal dry matter content of vegetable juice made of concentrate: they were 8, 7.5 and 10 (m/m)% for carrot, Jerusalem artichoke and beet, respectively. The strains showed as good growth and fermenting abilities in the scaling-up experiments (in 2-liter bench-top fermentor) as in flask scale experiments. The survival of the *B. lactis* Bb-12 strain in fermented products was investigated at 4°C. I found that the bifidobacterium as monoculture had kept its viability in carrot juice for 45 days. When Jerusalem artichoke juice was fermented with the mixed culture of *B. lactis* and *Lactobacillus casei* Shirota the bifidobacterium had kept its viability for 72 days. Cell concentration decreased one order of magnitude in carrot juice, while it remained in the order of the initial cell count in Jerusalem artichoke juice. The applied bifidobacterium strain showed good survival in Jerusalem artichoke juice at room temperature, and the cell count decreased only one order of magnitude after 72 days of storage. The other member of the mixed culture, the *Lb. casei* Shirota strain showed similar survival at both storage temperatures. My product development goals were achieved since it was possible to create such mixed starter culture composed of *B. lactis* Bb-12 and *Lb. casei* Shirota that fulfils requirements regarding both probiotics and the production technology.

Further possible direction of the research and development

- Elaboration of the mass propagation of some of the selected *Bifidobacterium* strains (own human isolates), production of starter culture and obtaining permission for its implementation in food processing.
- Purification, characterization of the antimicrobial substance produced by the *B. longum* A4.8 strain and exploration of its possible applications.
- Investigation of the biologically active components of fermented vegetable juices: highlighting the changes occurring during pretreatment and fermentation.
- Evaluation of the sensory properties of fermented vegetable juices and products, surveying consumer acceptance.

NEW SCIENTIFIC RESULTS

1. I have determined the effect of vancomycin antibiotics and hydrogen peroxide on the selected *Bifidobacterium* strains. I demonstrated that the strains were sensitive to vancomycin according to both by the agar diffusion method and in liquid culture. Vancomycin sensitivity of the individual strains in liquid culture was in the range of 0.75 to 3.0 μg/ml based on the value of minimal inhibitory concentration (MIC). The hydrogen peroxide tolerance of the bifidobacterium strains proved to be 375 μg/ml (MIC) determined by agar diffusion method, while the strains showed significant variation in liquid culture, independently of their species. In the latter case the MIC values were between 100 and 300 μg/ml. The *Bifidobacterium lactis* Bb-12 starter culture proved to be the most tolerant to hydrogen peroxide.
2. Growth of bifidobacteria is inhibited by melanoidins – products of Maillard-reaction – originating from both cocoa and bread. Degree of inhibition is strongly dependent of the molecular mass of the melanoidins, and the strains show diverse sensitivity to melanoidins of different origin.
3. I have elaborated a method for the determination of the selectivity of prebiotics. In the model system I have evaluated two commercially available prebiotics (Raftilose and Xylo-oligo 95P) with the paring of *B. lactis* Bb-12 and *E. coli* O157:H7 strains, and *B. adolescentis*^{T} and *E. coli* O157:H7 strains. I have determined that both the *B. lactis* Bb-12 and the *B. adolescentis*^{T} strain utilized the two prebiotics better than glucose, thus they may be regarded as bifidogenic factors. In the presence of Raftilose bifidobacteria reached higher cell concentration than the *E. coli* O157:H7 strain.
4. I have demonstrated that some *B. bifidum*, *B. longum* strain exert inhibition on the growth of *L. monocytogenes* 4ab, *Ec. faecalis, E. coli* O157:H7 and ATCC 8439 strains that is not the result of the effect of pH. I have determined that the growth conditions affect the degree of the antagonistic activity. In the growth medium of the strains showing antimicrobial activity I have found a 5-7 kDa size protein that inhibited the growth of *L. monocytogenes* 4ab.
5. I have demonstrated in a competitive adherence experiment that the adhering of *E. coli* Bay100 strain is inhibited by *B. lactis* Bb-12, but not inhibited by *B. bifidum* B3.2.
6. The examined plant raw materials – carrot, beet and Jerusalem artichoke juice – provide adequate nutritive materials for the growth of *B. lactis* Bb-12 starter culture. I have elaborated a mixed culture fermentation technology with the application of *B. lactis* Bb-12 and *Lb. casei* Shirota starter cultures. The strains stimulated the growth of each other indicating commensalist relation between them.

PUBLICATIONS ON THE SUBJECT OF DISSERTATION

Publications in scientific journals

Peer-reviewed articles in journals with impact factor

SZEKÉR K., CSIBRIK-NÉMETH E., **KUN SZ**., BECZNER J., GÁLFI P. (2007): Adhesion of lactic acid bacteria to Caco-2 cells-evaluation of different detection methods. *Acta Alimentaria*, 36 (3) 365-371. p.

SUMMA, C., MCCOURT J., CÄMMERER B., FIALA A., PROBST M., **KUN, SZ**., ANKLAM E., WAGNER K-H. (2008): Radical scavenging activity, anti-bacterial and mutagenic effects of Cocoa bean Maillard Reaction products with degree of roasting. *Molecular Nutrition and Food Research*, 52 (3) 342-351. p.

KUN, SZ., REZESSY-SZABÓ, JM., NGUYEN, DQ., HOSCHKE, Á. (2008). Changes of microbial population and some components in carrot juice during fermentation with selected *Bifidobacterium* strains. *Process Biochemistry*, 43 (8) 816-821. p.

Peer-reviewed article

KUN, SZ., ZALÁN, ZS., PERGER-MÉSZÁROS, I., HOSCHKE, Á. (2007): Mono- and mixed culture fermentation with *Lactobacillus* and *Bifidobacterium* strains on *Jerusalem artichoke* medium. *Hungarian Agricultural Research*, 16 (4) 10-13. p.

Proceedings in conference publications

KUN, SZ, MYSZOGLÁD, R., REZESSY-SZABÓ, J.M., HOSCHKE, Á. (2005): Implementation of soymilk in production probiotic food. Proceedings at Innovation and Utility in the Visegrad Fours, October 13-15, 2005, Nyíregyháza, Hungary, 461-466. p.

KUN, SZ., REZESSY-SZABÓ, J.M., NGUYEN, D.Q., HOSCHKE, Á. (2005): Antibacterial activity of *Bifidobacterium* strains. Proceedings at Intradfood2005, Innovations in Traditional Foods, October 25-28, 2005, Valencia, Spain, 501-504. p.

Abstracts in conference publications

In Hungarian

KUN SZ, REZESSY-SZABÓ JM, MAYER Á, HOSCHKE Á (2004): Probiotikus starterkultúrák alkalmazása szójaalapú termékek előállítására. MMT Nagygyűlés és X. Fermentációs Kollokvium. Keszthely, 2004. október 7-9. Összefoglaló 69.o.

BELÁK Á, KISKÓ G, MOHÁCSI-FARKAS CS, **KUN SZ**, REZESSY-SZABÓ J, MARÁZ A (2004): A kompetitív mikrobiota vizsgálata bifidobaktériummal erjesztett sárgarépalében. MMT Nagygyűlés és X. Fermentációs Kollokvium. Keszthely, 2004. október 7-9. Összefoglaló 10.o.

KUN SZ., NAGY Z., REZESSY-SZABÓ J.M., NGUYEN D.Q., HOSCHKE Á. (2005): Vegyes kultúrás erjesztések csicsókaporból készült tápközegben. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, Budapest, 2005. október 19-21, Összefoglaló 160.o.

KUN SZ., WAGNER K-H., REZESSY-SZABÓ J.M. (2005): Melanoidinek hatása a bifidobaktériumokra. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, Budapest, 2005. október 19-21, Összefoglaló 158.o.

SZEKÉR K., NÉMETH E., **KUN SZ**., BECZNER J., GÁLFI P. (2005): Tejsavbaktériumok tapadása Caco-2H sejtekhez-a fluoreszcencián alapuló detektálás lehetőségei. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, Budapest 2005. október 19-21, Összefoglaló 176.o.

KUN, SZ., PERGER-MÉSZÁROS, I., ZALÁN, ZS., REZESSY-SZABÓ, J.M. (2007): Vegyes bifido és LAB starter kultúra fejlesztése zöldség fermentációhoz. 328. Tudományos Kollokvium Biotechnológia szekció, Budapest, 2007. szeptember 28.

VIZI, T., **KUN, SZ.**, PERGER-MÉSZÁROS, I., HAVAS, P., REZESSY-SZABÓ, J.M (2007): Különböző baktérium törzsek szénhidrát hasznosítása. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, Budapest, 2007. november 7-8, Összefoglaló 102.o.

REZESSY-SZABÓ, J.M, KEREKES-MAYER Á., **KUN, SZ.**, PERGER-MÉSZÁROS, I., BUJNA E., NGUYEN, D.Q., DÜCSŐ, L., HOSCHKE, Á. (2007): Probiotikus termékek fejlesztési lehetőségei növényi eredetű nyersanyagokon. Regionális Élelmiszertudományi Kollokvium, Szeged, 2007 október 12.

REZESSY-SZABÓ, J.M, PERGER-MÉSZÁROS, I., **KUN, SZ.**, HOSCHKE, Á. (2007): Probiotikus termékek fejlesztése különleges táplálkozás igényű fogyasztók számára. Táplálkozástudomány Iskolája Konferencia, Budapest, 2007. november 23.

In a foreign language

KUN, SZ., REZESSY-SZABÓ, J.M., MAYER, Á., NGUYEN, D.Q., HOSCHKE, Á. (2004):Cultivation of probiotic *Bifidobacterium* strains in synthetic and natural media. 2nd Central European Congress on Food, April 26-28, 2004, Budapest, Hungary, 143. p.

KUN, SZ., MAYER, Á., REZESSY-SZABÓ, J.M., NGUYEN, D.Q., HOSCHKE, Á. (2004): Possibilities for production of non-conventional probiotic food. FoodMicro 2004, September 12-16, 2004, Portoroz, Slovenia, 385. p.

SZEKÉR, K., NÉMETH, E., **KUN, SZ**., BECZNER, J., GÁLFI, P. (2005): Adhesion of lactic acid bacteria to Caco-2H cells – possibilities for detection. 1st Central European Forum for Microbiology (CEFORM), October 25-28, 2005, Keszthely, Hungary, 155. p.

KUN, SZ., REZESSY-SZABÓ, J.M., NGUYEN, D.Q., HOSCHKE, Á. (2006): Organic acid production by beneficial bifidobacteria in carrot juice. The SAFE Consortium International Congress on Food Safety, June 11-14, 2006, Budapest, Hungary, 28. p.

KUN, SZ., DALMADI, I., DÜCSŐ, L., HOSCHKE, Á. (2006): Application of high pressure processing for pre-treatment of carrot juice prior to fermentation. FoodMicro2006 (food safety and food biotechnology), August 29-September 2, 2006, Bologna, Italy, 485. p.

PERGER-MÉSZÁROS, I., ZALÁN, ZS., **KUN, SZ**., REZESSY-SZABÓ, J.M. (2007): Development of vegetable based probiotic food applying mixed culture. 4th Probiotics, Prebiotics & New Food, September 16-18, Rome, Italy, Cibus, Vol: 3, 84 p.

VIZI, T., **KUN, SZ**., NGUYEN, D.Q., REZESSY-SZABÓ, J.M (2007): Utilization of prebiotics by various bacterial strains. 15th International Congress of the Hungarian Society for Microbiology, July 18-20, Budapest, Hungary. Abstract in Acta Microbiol. Immunol. Hung., 54, S143-S144.