

VIABILITY OF MICROFLORA OF MARKET FERMENTED MILK PRODUCTS IN SIMULATED CONDITIONS OF GASTRIC AND DUODENUM*

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Background. The non-probiotic lactic acid bacteria have only rarely been used in *in vitro* or *in vivo* studies, because they are not considered to exert health benefits. To exert the beneficial effect in human organism, LAB needs to meet some criteria, for example the viability in the gastrointestinal tract (GIT). The aim of this work was to determine the viability of microflora of chosen market non-probiotic fermented milk products in simulated gastric and duodenal fluids.

Material and methods. Ten market non-probiotic fermented milk products bought in Warsaw were used in this study. Ten grams of each product have been suspended in simulated gastric fluid, and then the mixture has been transferred into simulated duodenal fluid. Immediately after bacteria inoculum addition and at the end of the experiment, the number of lactobacilli and lactococci was measured.

Results. The number of lactococci or streptococci decreased by 0.1-0.3 log cycle after 3 h in gastric mixture. Only in one yoghurt the population of streptococci decreased by 0.9 log cycle. The population of lactobacilli did not change in condition of simulated gastric fluid. The significant reduction of lactobacilli, lactococci and streptococci population was observed after the transfer of mixture into simulated duodenal fluid and incubation in this condition for 5 h. After the end of experiments in every studied sample the number of microflora remained at the level above 6 log CFU/mL.

Conclusions. The results indicate that the condition simulating gastric fluid is not a menace to viability of lactic acid bacteria, if they are protected by milk products. The significant reduction of bacteria number in simulated duodenal fluid was probably caused by cell shock to intensive pH change of environment and loss of protective barrier caused by digestive enzymes activity.

Key words: lactic acid bacteria, LAB, gastrointestinal tract, survival, simulated gastric fluid, simulated duodenal fluid, fermented milk products

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INTRODUCTION

In modern fermented milk products manufacture bacteria of the group commonly referred to as lactic acid bacteria (LAB) are added to milk as starter cultures, the key role being the production of lactic acid by fermentation of lactose. The bacteria most widely used in the manufacture of fermented dairy products are generally lactic acid bacteria of four genera: *Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Leuconostoc* [Varnam and Sutherland 2001, Leroy and De Vuyst 2004]. *Streptococcus thermophilus* is the only species of *Streptococcus* genus found in dairy starter cultures. Bacteria of *Lactobacillus* genera are also used as dairy starters in the manufacture of yoghurt, and Mozzarella cheese [Gardiner et al. 1999, Varnam and Sutherland 2001, Leroy and De Vuyst 2004]. They are also used to promote faster ripening of Cheddar and similar cheeses. *Lactobacillus delbrueckii* subsp. *bulgaricus* is widely used along with *S. thermophilus* as a starter in yoghurt manufacture. This subspecies is thermophilic (has an optimum temperature of 42°C and grows at temperatures of 45°C and higher). In comparison, *Lb. acidophilus* and *Lb. casei*, which are normally present in the intestine, are generally not used as a starter but as a probiotic in fermented milk products manufacture.

Lactic acid bacteria are beneficial to human health and thus some strains of them are probiotics, like some strains of *Lb. acidophilus*, *Lb. casei* or *Bifidobacterium* sp. To exert the beneficial effect in human organism, LAB needs to meet to some criteria. But *Lactococcus lactis*, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* have only rarely been used in *in vitro* or *in vivo* studies, because they are not considered to exert health benefits. Meanwhile the survival in the gastrointestinal tract (GIT) could be one of these criteria [Conway et al. 1987, Gardiner et al. 1999, Lick et al. 2001, Oozeer et al. 2002]. The main factors influencing the survival of LAB in GIT are: low pH in the stomach, and intestinal peristalsis, bile salts and different digestive enzymes present in the duodenum (the first section of the small intestine) [Marteau et al. 1997, Elli et al. 2006, Sharp et al. 2008]. The gastric fluid is one of the main secretions of the stomach, together with several enzymes and intrinsic factor. It is an acid solution with a pH of 1 to 2, consisting mainly of hydrochloric acid, small quantities of potassium chloride and sodium chloride. It had a high pepsin concentration. The duodenal fluid contains many enzymes (proteolytic enzymes, glucoamylases, oligo-1,6-glucosidases, saccharases, maltases, lactases and lipases) produced by the pancreas, and bile salts secreted by the liver [Brigidi et al. 2003, Michajlik and Ramotowski 2003]. The pH of the pancreatic fluid is 7.0-8.7 and the pH of the duodenal fluid is 6.5-7.5. Additionally, in the duodenum, there are Brunner glands secreting the mucus at pH 8.3-9.3, that neutralize the acidity of gastric fluid and protect the duodenum before low pH of the content inflowing from the stomach.

Lactic acid bacteria have the potential effect on improving human health. The knowledge about factors affecting viability of these microorganisms in conditions of gastrointestinal tract maybe contributes to use them as probiotics. There are few research studies on non-probiotic lactic acid bacteria in GIT and there are conflicting results concerning their survival in GIT. There are some substances or factors having a protective activity on LAB and enhancing their survival during the passage through GIT. The foodstuffs, proteins and fats especially, have protective effects on the bacterial cells [Ibrahim and Bezkorovainy 1993, Drouault et al. 1999, Lick et al. 2001].

The aim of this work was to determine of viability of microflora of chosen market non-probiotic fermented milk products in simulated gastric and duodenal.

MATERIAL AND METHODS

Materials. Ten market fermented milk products (3 yoghurts, 1 kefir, 1 buttermilk, 1 curdled milk, 1 yoghurt soured cream, 2 probiotic homogenised cheeses, and 1 liquid probiotic milk base drink) bought in Warsaw market was used in this study. The study involved microbiological determinations of survival of microflora of chosen market fermented milk products in simulated gastric and duodenal fluids. No probiotics were declared on the label of all products except liquid probiotic milk base drink which contained probiotic strain of *Lb. casei*. All products were used in the experiments immediately after purchasing, before the expiration date. Yoghurts contained av. 3.5 g of protein, 3.3 g of fat, and 4.7 g of carbohydrates per 100 g. Kefir contained 2.6 g of protein, 2 g of fat, and 3.8 g of carbohydrates per 100 g. Buttermilk and curdled milk contained av. 3.3 g of protein, 1 g of fat, and 4.8 of carbohydrates per 100 g. Yoghurt soured cream contained 12% of fat. Probiotic homogenized cheeses and liquid probiotic milk base drink contained 16.8 g and 2.8 g of protein, 4.2 g and 1.3 g of fat, and 13.2 g and 10.5 g of carbohydrates, respectively.

Artificial gastric fluid. Artificial gastric fluid was prepared by supplementing basic gastric fluid with pepsin. The basic gastric fluid was prepared according to Clavel et al. [2004] with some modifications. It contained 4.8 g of NaCl (POCH, Poland), 1.56 g of NaHCO₃ (POCH, Poland), 2.2 g of KCl (POCH, Poland) and 0.22 g of CaCl₂ (POCH, Poland) dissolved in 1 L of distilled water. After autoclaving at 121°C/15 min, the pH of the basic gastric fluid was adjusted to 2.4 ±0.2 using 1 M HCl, and 2 mg of pepsin (Sigma Aldrich, USA) was added per 50 mL of the artificial gastric fluid.

Artificial duodenal fluid. Artificial duodenal fluid was prepared by supplementing the basic duodenal fluid with the enzyme complex. The basic duodenal fluid was prepared according to Marteau et al. [1997] with some modifications. It contained 5.0 g of NaCl (POCH, Poland), 0.6 g of KCl (POCH, Poland), 0.03 g of CaCl₂ (POCH, Poland) and 17 g of bile salts (Merck, Germany) dissolved in 1 L of 1 mol/L NaHCO₃ (POCH, Poland). After autoclaving at 121°C/15 min, the pH of the basic juice was 7.0 ±0.2 using 1 M NaOH, and the enzyme complex was added (two capsules per 50 mL of fluid). The pharmaceutical preparation called Kreon® 10000 (Solvay Pharmaceuticals, USA) was used as the source of the enzyme complex. One capsule of Kreon® 10 000 contains 150 mg of pancreatic enzymes: 10,000 F.I.P. units of lipases, 8000 F.I.P. units of amylases, and 600 F.I.P. units of proteases.

The experiments. Ten grams of each product were suspended in 50 mL simulated gastric fluid for 3 h at 37°C. Next the mixture was transferred into 50 mL simulated duodenal fluid for 5 h at 37°C. Immediately after bacteria inoculums addition and at the end of experiment, the number of lactobacilli and lactococci were measured. The number of lactococci was measured using classical plate method and M17 agar (Merck, Germany). Plates were incubated aerobically at 30°C/72 h (mesophilic lactococci) or at 37°C/72 h (thermophilic streptococci). The number of lactobacilli was measured using MRS agar (Merck, Germany). Plates were incubated anaerobically at 37°C/72 h. Each product was tested in three replicates, then the mean and SD were calculated.

Statistical analysis. Statistical analysis (ANOVA and Tukey test) between the initial and final microbial counts was performed using the Statgraphics Plus 5.1 software.

RESULTS

The bacteria widely used in the manufacture of fermented dairy products are generally non-probiotic lactic acid bacteria. To exert their beneficial effect in human organism, lactic acid bacteria need to survive in the gastrointestinal tract. As mentioned before, to health benefits, they must express high tolerance to acid, bile salts, and be able to bind to the epithelial cells of GIT. In this work, studied microflora of fermented milk products showed variable ability to survive in simulated conditions of gastric and duodenum (Table 1 and 2).

Table 1. The count of lactococci cells in the gastric and duodenal fluids (mean and standard deviation of three determinations)

| Product | 3 h cultures in gastric fluid | | 5 h cultures in duodenal fluid | |
|----------------------------------|-------------------------------|---------------------------|--------------------------------|---------------------------|
| | initial count log CFU/mL | final count log CFU/mL | initial count log CFU/mL | final count log CFU/mL |
| Yoghurt 1 | 8.0 ±0.2 a | 7.9 ±0.2 a | 7.6 ±0.2 a | 5.8 ±0.4 b |
| Yoghurt 2 | 8.3 ±0.2 a | 8.0 ±0.5 a | 7.7 ±0.5 a | 4.8 ±0.1 b |
| Yoghurt 3 | 8.0 ±0.1 a | 7.1 ±0.5 a | 6.8 ±1.0 a | 2.9 ±0.1 b |
| Liquid probiotic milk base drink | 7.9 ±0.3 a | 7.8 ±0.1 a | 7.5 ±0.1 a | 5.4 ±1.2 b |
| Yoghurt soured cream | 7.7 ±0.8 a | 7.5 ±0.9 a | 7.2 ±0.9 a | < 2.0 b |
| Probiotic homogenized cheese 1 | 7.4 ±0.3 a | 7.3 ±0.4 a | 6.8 ±0.3 a | 3.7 ±1.5 b |
| Probiotic homogenized cheese 2 | 6.3 ±0.9 a | 6.2 ±1.3 a | 5.8 ±1.5 a | < 2.0 b |
| Curdled milk | 6.4 ±0.6 a | 6.1 ±0.2 a | 5.7 ±0.4 b | 4.1 ±1.3 c |
| Kefir | 7.7 ±0.7 a | 7.4 ±1.0 a | 7.0 ±0.9 a | < 2.0 b |
| Butter milk | 6.7 ±0.7 a | 6.5 ±0.2 a | 6.0 ±0.2 a | 3.5 ±1.2 b |

Different letters (a, b, c) in the same row indicate statistically significant differences ($p < 0.05$).

Table 2. The count of lactobacilli cells in the gastric and duodenal fluids (mean and standard deviation of three determinations)

| Product | 3 h cultures in gastric fluid | | 5 h cultures in duodenal fluid | |
|----------------------------------|-------------------------------|---------------------------|--------------------------------|---------------------------|
| | initial count log CFU/mL | final count log CFU/mL | initial count log CFU/mL | final count log CFU/mL |
| Yoghurt 1 | 7.2 ±0.1 a | 7.2 ±0.3 a | 6.9 ±0.2 a | 3.2 ±0.5 b |
| Yoghurt 3 | 6.6 ±0.2 a | 6.3 ±0.3 | 6.1 ±0.1 | 2.7 ±0.1 |
| Liquid probiotic milk base drink | 7.7 ±0.1 a | 7.8 ±0.1 a | 7.3 ±0.4 a | 5.8 ±1.3 b |
| Yoghurt soured cream | 7.0 ±0.9 a | 7.0 ±0.9 a | 6.8 ±0.9 a | 3.4 ±0.1 b |
| Probiotic homogenized cheese 1 | 6.7 ±1.4 a | 6.1 ±1.5 a | 5.7 ±0.1 b | 2.6 ±0.1 c |
| Probiotic homogenized cheese 2 | 6.5 ±1.0 a | 7.0 ±0.9 a | 6.7 ±0.1 a | 3.8 ±0.1 b |
| Butter milk | 6.1 ±0.3 a | 6.1 ±0.4 a | 5.6 ±0.1 b | 2.7 ±0.4 c |

Different letters (a, b, c) in the same row indicate statistically significant differences ($p < 0.05$).

The initial number of lactococci or streptococci in the studied fermented products was in range from 6.1 log CFU/g to 8.9 log CFU/g, and decreased to 6.3-8.3 log CFU/mL due to the dilution by the addition to simulated gastric fluid. The reduction of lactococci or streptococci population in simulated gastric fluid was observed in every sample, despite initial pH value of this fluid was ca. 2.5. After 3 h of incubation in gastric mixture the number of lactococci or streptococci decreased to 6.1-8.0 log CFU/mL (Table 1). Only in one yoghurt (No 3) the population of streptococci decreased by 0.9 log cycle. According to the statistical analysis, the reduction of lactococci or streptococci population was significant after the transfer of gastric mixture into simulated duodenal fluid (initial pH was 7.0 \pm 0.2) and incubation in this condition for 5 h ($p = 0.0000$). The number of lactococci or streptococci population decreased from initial 5.8-7.7 log CFU/mL to final 2.9-5.8 log CFU/mL, and depended on the type of products ($p = 0.0000$). Only the microflora from three products was below 2.0 log CFU/mL (yoghurt soured cream, probiotic homogenised cheese, and kefir). The microflora of three yoghurt samples, probiotic homogenised cheese No 1, and probiotic milk base drink survived much better than microflora of other fermented milk products. After the end of experiments in every studied sample the number of lactococci or streptococci remained at level above 6.0 log CFU/mL.

In the present work, lactobacilli were present in seven samples (in number ranged from 6.0 log CFU/g to 8.7 log CFU/g, in one yogurt sample lactobacilli were absent) and after the transfer to simulated gastric fluid their initial number ranged from 6.7 to 7.7 log CFU/mL. The population of lactobacilli did not change in simulated gastric fluid (Table 2). The final number of lactobacilli after the incubation in simulated gastric fluid was 6.1-7.8 log CFU/mL. The significant reduction of lactobacilli population was observed after the transfer of gastric mixture into simulated duodenal fluid and incubation in this condition for 5 h. The number of lactobacilli decreased significantly by 1.5-3.7 log ($p = 0.0001$), from initial 5.6-7.3 log CFU/mL to final 2.6-5.8 log CFU/mL. The reduction of lactobacilli population did not depend on the type of products, except the microflora of liquid probiotic milk base drink, which survived better than bacteria from other products.

DISCUSSION

Lactococcus lactis subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are mainly used for production of traditional milk products: fresh cheeses, soured cream, curdled milk, butter milk, kefir, and yoghurt. The concentration of these organisms in the human or animal gastrointestinal tract has been poorly examined in comparison with that of other probiotic strains. In the papers, there are conflicting results concerning the survival of non-probiotic LAB in GIT, especially *Lactococcus* species. There have been few studies on the probiotic properties of bacteria *Lactococcus*, since they are not natural inhabitants of GIT. Some authors reported that non-probiotic LAB did not survive the passage through the intestinal tract, and was not recovered from faeces of humans after daily yoghurt ingestion [Pedrosa et al. 1995, Vesa et al. 2000, Brigidi et al. 2001, Kimoto et al. 2003, Del Campo et al. 2005, Mater et al. 2005]. Vesa et al. [2000] demonstrated that *L. lactis* survived only at level 1% \pm 0.8 in the duodenum. Kimoto et al. [2003] recovered noted that viable cells of *Lactococcus*

lactis subsp. *lactis* biovar. *diacetylactis* N7 from faeces within 24–48 h after administration to mice but not at 72 h. Other lactococci strain studied by Kimoto et al. [2003], *L. lactis* subsp. *cremoris* ATCC 19257, which had a poor survival rate *in vitro* test, was also detected at 12 h but not at 24 h.

There have also been conflicting studies concerning the survival of yoghurt bacteria (*S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) in gastrointestinal tract. The conflicting results found in the papers towards the survival of bacteria in gastrointestinal tract maybe are linked the different methodologies performed as well the chemical composition of food matrix chosen [Gardiner et al. 1999]. Lick et al. [2001] studied the ability of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* administered in yogurt to survive the passage through the upper gastrointestinal tract was investigated with Gottingen minipigs. After ingestion of yogurt containing viable microorganisms, living *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were detected in the magnitude of 10^6 to 10^7 per gram of intestinal contents in all animals under investigation. Elli et al. [2006] confirmed that yoghurt bacteria, especially *Lb. delbrueckii* subsp. *bulgaricus*, can be retrieved from faeces of healthy individuals after a few days of ingestion of commercial yoghurt. It could suggest that there are some undefined factors influencing the ability of LAB to survive in duodenal fluid. Moreover, other LAB, for example *Lb. acidophilus* and *B. bifidum*, has the ability to establish *Lb. delbrueckii* subsp. *bulgaricus* among the gut flora [2000]. It is obvious that the survival of yoghurt bacteria depends on the dose of bacteria cells (or products containing bacteria) introduced in gastrointestinal tract. In the present work the dose of product in simulated gastric fluid was 10 g per 50 mL of fluid. It suggest that another doses of product may have different influence on the survival of yoghurt bacteria in gastrointestinal fluids.

Some authors reported also that *S. thermophilus* were not recovered from faeces of subjects [Pedrosa et al. 1995, Del Campo et al. 2005, Elli et al. 2006]. Brigidi et al. [2003] recovered *S. thermophilus* from faecal samples for 6 days after the end of intake of a pharmaceutical preparation orally for 3 days. The persistence of a yoghurt culture in the human gut was also confirmed by Lick et al. [2001] and Mater et al. [2005].

Conway et al. [1987] also showed that the ability of LAB to survive in GIT varies according to the species. In the present work the same destructive effect of simulated duodenal fluid on lactococci, streptococci or lactobacilli population has been found. The significant reduction of bacteria number in simulated duodenal fluid has been probably caused by cell shock on intensive pH change of environment and loss of protective barrier caused by digestive enzymes activity on food.

The ability of LAB to survive passage through GIT is mainly attributed to their acid in the stomach and bile tolerance in the duodenum. Chemically, gastric fluid is an acid solution, which is neutralized in the duodenum by sodium bicarbonate. This also blocks gastric enzymes that have their optima in the acid range of pH. The duodenum is mainly responsible for the breakdown of food in the small intestine. Marteau et al. [1997] presented the dynamic model of the gastrointestinal tract, with the simulation of peristalsis, the changes in pH, the changes in concentration of the enzymes and bile salts. They explored the survival of same strains of lactic acid bacteria. They found that survival of LAB strains in applied dynamic model was compared with data obtained from human, *in vivo*. The bile salts are the serious barrier for the LAB, because of the presence of bile acids toxic for bacterial cells [Kailasapathy and Chin 2000, Bezkorovainy 2001]. Lankaputhra and Shah [1995] observed different survival of studied strains of *Lactobacillus* suspended in the medium contained from 0 to 1.5% of bile salts. Vinderola and

Reinheimer [2003] found that *Lb. casei* did not deconjugate the bile salts, as to other species of the *Lactobacillus* genera did, but it was resistant against bile salts. Sharp et al. [2008] showed that low-fat Cheddar cheese is a viable delivery food for probiotic strain of *Lb. casei* because it helped protect cells against the very low pH (in 8.7 mM phosphoric acid at pH 2, 37°C for 30 min) that will be encountered during stomach transit.

The present results indicate that the condition simulating gastric fluid is not a menace to viability of lactic acid bacteria, if they are protected by milk compounds. This work confirmed the results obtained by Pochart et al. [1992] and Drouault et al. [1999] suggesting that food may have an important protective effect on the bacteria present in diet. Pochart et al. [1992] demonstrated that in healthy adults *Bifidobacterium* sp. survived transit through the gastrointestinal tract when ingested in fermented milk. Drouault et al. [1999] showed that the cells of *L. lactis* mixed with food survived much better in the duodenum than pure *L. lactis* cultures. *Lactococcus* which transit with the diet were quite resistant to gastric acidity (90-98% survived). In contrast, only 10-30% of bacteria cells survived in the duodenum. Viable cells were metabolically active in each compartment of the digestive tract, whereas majority of dead cells appeared to be subject to rapid lysis.

Ziarno and Margol [2007] determined the ability of selected pure LAB cultures to survive in a simulated gastric fluid and in a culture broth. They proved that the probiotic strains, obtained from chosen dairy starter cultures, had better viability in the simulated gastric fluid than the traditional starters (present in commercial dairy starter cultures such as yoghurt starters).

Ziarno [2007] demonstrated that survival rate of pure cultures of eight species of LAB (*Bifidobacterium*, *Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. plantarum*, *Lb. casei*, *Lb. rhamnosus*, *L. lactis*, *S. thermophilus*) in simulated duodenal fluid depended on the initial count of bacteria. Pure *Lb. delbrueckii* subsp. *bulgaricus* cultures survived better in artificial duodenal fluid than studied bifidobacteria and similar to *Lb. acidophilus* strains. The initial count of *Lb. acidophilus* isolates ranged from 4.6 to 7.5 log CFU/mL, and after culturing in artificial duodenal fluid decreased to 2.3-5.2 log CFU/mL. In comparison, the mean survival of bifidobacteria in simulated duodenal fluid ranged from 0% to 50.0% of initial log of bacteria number. Pure *L. lactis* subsp. *lactis* culture studied by Ziarno [2007] showed good resistance to artificial duodenal fluid and the mean survival was 58.3% of initial log of bacteria number. Pure *S. thermophilus* culture survived in simulated duodenal fluid, as well as bifidobacteria isolates did, and mean survival rate was 45.7% of initial log of bacteria number and depended on the initial count of bacteria.

In conclusion, lactic acid bacteria able to survive the low pH values of the stomach and to tolerate the bile salts in the duodenum could be potential probiotic bacteria. First survival tests for potential probiotic strains could be performed *in vitro*. Dunne et al. [2001] showed that *in vitro* study of LAB can result in the isolation of strains capable of performing effectively in the gastrointestinal tract. Due to their simplicity, *in vitro* studies using simulated conditions of gastric and duodenum appeared to be suitable model systems for the screening of the survival of LAB in human gastrointestinal tract. In the present study, lactic acid bacteria mixed in market fermented milk products showed quite good resistance to simulated gastric fluid, and only in simulated duodenal fluid their population has been reduced significantly. It is worth to stress that lactococci or streptococci survived in simulated conditions of gastric and duodenum, as well as the lactobacilli.

CONCLUSIONS

1. The survival of studied microflora of chosen fermented milk products in simulated conditions of gastric and duodenum does not depend on the type of product.
2. The significant reduction of lactobacilli, lactococci and streptococci population has been observed in simulated duodenal fluid (initial pH was 7.0 ± 0.2) not in simulated gastric fluid (initial pH was 2.4 ± 0.2).
3. The condition simulating gastric fluid is not a menace to viability of lactic acid bacteria, if they are protect by milk products.
4. Lactococci and streptococci survive in simulated conditions of gastric and duodenum as well as the lactobacilli.

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PRZEŻYWALNOŚĆ MIKROFLORY HANDLOWYCH MLECZNYCH PRODUKTÓW FERMENTOWANYCH W SYMULOWANYCH WARUNKACH ŻOŁĄDKA I JELIT

Wprowadzenie. Nieprobiotyczne bakterie mlekowe rzadko bywają przedmiotem badań *in vitro* lub *in vivo*. Uważa się, iż nie przynoszą one ludzkiemu organizmowi korzyści prozdrowotnych. Musiałyby spełniać określone kryteria, np. przeżywalność w warunkach układu pokarmowego. Celem pracy było określenie przeżywalności mikroflory wybranych rynkowych nieprobiotycznych fermentowanych produktów mlecznych w symulowanych sokach żołądkowym i trzustkowym.

Materiał i metody. Materiałem do badań było 10 nieprobiotycznych mlecznych produktów handlowych z rynku warszawskiego. Dziesięć gramów każdego produktu zostało zawieszonych w symulowanym soku żołądkowym, a następnie mieszaninę przenoszono do symulowanego soku trzustkowego. Bezpośrednio po zawieszeniu bakterii w soku i po zakończeniu hodowli oznaczano liczbę pałeczek mlekowych oraz paciorkowców mlekowych.

Wyniki. Liczba komórek paciorkowców lub streptokoków zmniejszyła się o 0,1-0,3 log po 3 h inkubacji w mieszaninie żołądkowej. Tylko w jednym jogurcie populacja paciorkowców zmniejszyła się o 0,9 log. Populacja pałeczek mlekowych nie zmieniła się istotnie w warunkach symulowanego soku żołądkowego. Po przeniesieniu mieszaniny do symulowanego soku trzustkowego, o początkowej wartości pH $7,0 \pm 0,2$, oraz inkubacji w tych warunkach przez 5 h, w żadnym z badanych produktów liczba mikroflory nie utrzymała się na poziomie ponad $6 \log \text{ jtk/cm}^3$ po zakończeniu inkubacji w symulowanych sokach żołądkowym i trzustkowym.

Wnioski. Środowisko symulujące warunki panujące w żołądku nie jest zagrożeniem dla żywotności bakterii mlekowych, jeśli są one podawane wraz z pokarmem chroniącym je przed niskim pH soków trawiennych. Z kolei w symulowanym soku trzustkowym znaczna redukcja liczby komórek bakterii prawdopodobnie jest wywołana szokiem komórek na gwałtowną zmianę wartości pH środowiska i utratą ochronnej bariery pokarmowej wynikającą z działania enzymów trawiennych.

Słowa kluczowe: bakterie fermentacji mlekowej, LAB, układ pokarmowy, przeżywalność, sztuczny sok żołądkowy, sztuczny sok trzustkowy, fermentowane produkty mleczne

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