

Article

Effects of the complex food matrices on viability of probiotic cells during model digestion

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Abstract: The aim of this work was to evaluate the influence of various food and beverages on the viability of probiotic bacteria during passing through artificial digestion. As a model food, solutions with various concentrations of alcohol, sugar, salt, protein and acid were prepared. Different types of real foods and beverages were used as well. Viability in presence of food matrices was tested on monocultures of *Lactobacillus acidophilus* CCM4833 and *Bifidobacterium breve* CCM7825T and on mixed commercial culture with 9 different strains of probiotic microorganisms (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*). The concentration and viability of probiotic bacteria was tested by cultivation assay and flow cytometry. In model foods, the best growth of was determined in the presence of 10% albumin and 10% ethanol. Survival of the probiotics delivered in different food matrices through a simulated gastrointestinal tract was quantitatively different. As the best food environment for probiotics complex food matrices such as pasta with cream sauce, chocolate spread and homemade beef broth were selected, followed by mixed vegetables, potato salad, salted chips, fruits and yoghurt. Among beverages the best option was milk, followed by black tea, coffee and Coca Cola. Probiotic microorganisms are more viable when consumed with meals than with beverages only. In general, the highest viability of probiotic cells has been observed in presence of foods containing high concentration of sugar and fat or their suitable combination. The increase of cell viability observed in such foods during model digestion may further contribute to the positive effect of probiotics on human health.

Keywords: Probiotics; food matrices; cell viability; model digestion;

1. Introduction

Probiotics are living microorganisms that, when administered in sufficient quantity (around 10^6 – 10^7 CFU/mL or g of carrier food product) in provide a health benefit to the host, in particular through a replacement or inclusion process beneficial bacteria in the gastrointestinal tract [1,2].

In many studies the effects of probiotics and also prebiotics on various diseases was observed. For example, it was confirmed that dietary fiber and probiotics have positive effects on infectious diseases [3]. Probiotics have also shown positive responses to clinical treatment against several diseases and disorders, such as constipation and diarrhea, food allergies, inflammatory bowel disease, preventing and treatment of diabetes, obesity and cancer and diseases related to pathogenic microbes [4-6]. The antimicrobial activity of probiotics occurs through i) reduced pH due to production of acetic and

lactic acids, ii) bacteriocins accumulation and iii) compounds blocking bacterial adhesion to the epithelial cells and consequently reducing pathogen toxins production [7]. Probiotic bacteria are crucial for the maturation of immune cells too. This intestinal microbiota stimulates the maturation and functionality of the immune cells through their metabolites [5]. Also, probiotics are involved in regulation of intestinal health, improved lactose digestion and maintaining bone health and they make functional components such as antioxidants and anti-hypertensive [7]. Further, recent evidence and ongoing studies suggest that intestinal microbiota has a bidirectional effect on mood disorders and can thus effect on stress and anxiety [4,8]. Research on human diseases is revealing the vital roles played by the gut microbiota. Understanding the impact of the gut microbiota on the host health is essential to design strategies focused in probiotics manipulation. So, for example other knowledge on options which are responsible for increase of viability of probiotic will allow us to design new strategies to improve the health of the consumer [9].

There are several mechanisms, by which probiotics may benefit human, including production of antimicrobial substances, strengthening of intestinal barrier, modulation of immune response, and antagonism of pathogenic microorganisms either by production of antimicrobial agents or by competition for binding sites, nutrients, and growth factors [4,10,11]. More precisely, the interaction between probiotics and pathogens can be categorized in three steps: the physical interaction between the probiotic and the epithelium, the interaction between probiotics and the immune system and, finally, direct interaction between probiotics and pathogens [12]. The interaction between probiotics and pathogens may be observed in the hosts, but also in foods where can have a positive effect too and incorporating of probiotics in food matrices can be new option for food safety [11,12]. Moreover, probiotic bacteria incorporated into foods should be able to survive gastric transit and reach the small intestine in sufficient numbers of viable cells [2,11].

Food substrate is considered as one of the major factors in regulating colonization of probiotics in gastrointestinal tract. Food helps to buffer the bacteria through the stomach and may contain other functional ingredients, for example prebiotics, that can influence growth, viability and survival, acid and bile tolerance, adhesion of probiotics to intestinal cells [11] and different functionality of probiotics that determine their efficacy in gastrointestinal tract [13].

A way of increasing the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics, which provide an improved survival during the passage of the probiotic bacteria through the intestinal tract [14]. Development of functional foods also may to modulate gut microbiota and convey health effects. Major challenge in this area can be the incorporation of probiotics in foods, selection of the prebiotic candidate or selection of bacterial strains and encapsulation of probiotic bacteria [8]. However, the addition of prebiotics to products may negatively influence the product. Also addition of probiotics may change the sensory quality of product, because some strains may grow in the food matrix and producing metabolites, which interact with the food. The encapsulation of the probiotic may also affect the food texture [15]. On the other hand, dietary supplements in the form such as capsules, tablets and other formats may be used. But a current study on probiotics does not present a definitive answer as to whether there is superiority or equivalence on delivery of probiotics in foods or in the form of supplement [10].

However, probiotics which are used as supplements may reduce functional efficacy of probiotics due to exclusion of the potential synergistic effect of the food if they are not served together with appropriate food [13]. Many studies have focused on testing the effect of food matrix on probiotics during storage. Regarding the incorporation of probiotics into food products, the food matrix should

meet this requirements: low aw; neutral or slightly acidic pH, presence of fiber or prebiotic compounds and a high buffering capacity (for example a high fat content). For incorporation of probiotic strains into the product is also important the strain selection [16]. But the effect of food on probiotics during digestion must be also tested, because the ability to survive in the gastrointestinal environment is recognized as a fundamental requisite for probiotics [17].

The main goal of this work was a study the influence of different type of food and beverages on the viability and growth of probiotic bacteria during passing through the gastrointestinal tract. The results can be used as a basis for recommendation of dietary regime to optimum probiotics intake. To authors best knowledge, similar study of viability of probiotics in the presence of such diverse real foods and beverages during simulated digestion was not published yet.

2. Materials and Methods

2.1 Material

2.1.1 Probiotic strains

Bacterial strains *Lactobacillus acidophilus* CCM 4833 and *Bifidobacterium breve* CCM 7825T used in this study were purchased from Czech Collection of Microorganisms in Brno, Czech Republic.

The commercial preparation Biopron 9 manufactured by Walmark was used in this work. This food supplement contained a mixture of 9 bacterial cultures of *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus lactis* and *Streptococcus thermophilus*, 9×10^9 CFU (colony-forming units) in daily dose.

2.1.2 Food matrices

All used samples of real foods are summarized in **Table 1**. Beverages and instant foods were obtained from local retail. Home-made products were prepared immediately before experiments.

Table 1. Overview of tested real foods and beverages

| Beverages | Soups | Food - main course | Food - snacks | |
|--------------|---|-----------------------------------|----------------------------------|--------------------------------------|
| water | beef broth | hamburger | yoghurt chocolate | porridge |
| black tea | pea instant soup with croutons | instant pasta with cream sauce | pudding with whipped cream | poppy seed cake |
| black coffee | instant soup with liver dumplings | chicken with rice | chocolate spread | pastry |
| beer | vegetable broth - homemade | | potato salad | apple with banana |
| Juice | chicken broth - homemade | | chips - salted | tomato, cucumber and white pepper |
| milk | | | | |
| coca-cola | beef broth - homemade | | | |

2.2 Methods

2.2.1 Cultivation of probiotic bacteria

The strains were inoculated into MRS broth (HiMedia, India) and incubated at 37 °C for 48 h. The cells were harvested by centrifugation at 6000 rpm, 4 °C for 10 min (Hermle Z36 HK, Hermle, Germany) and washed with distilled water. Afterwards, cells were used for analysis.

Before use, the probiotic strains from the commercial Biopron capsule were hydrated in sterile water for 20 minutes and then used for inoculation and cultivation.

2.2.2 Model food matrices

As model food matrices, solutions with various concentrations of alcohol (ethanol), sugar (glucose), salt, protein (bovine serum albumin) in solutions of different pH were prepared. Standard and pure chemicals were purchased from Sigma Aldrich (Germany). All used model foods are summarized in **Table 2**.

Table 2. Overview of tested model foods

| | 1 | 2 | 3 | 4 |
|---------------------------------|---------|-----------------|--------|--------|
| Sterile distiller water | pH 7 | pH 3 (with HCl) | | |
| Protein (Bovine albumin) | 5 g/L | 10 g/L | 20 g/L | |
| Saccharide (Glucose) | 0.2 g/L | 2 g/L | 10 g/L | |
| Salt (NaCl) | 0.4 g/L | 1.2 g/L | 4 g/L | 10 g/L |
| Alcohol (Ethanol) | 5% | 10% | 20% | 40% |

2.4 Model digestion

Artificial stomach juice was prepared from 0.25 g of pepsin dissolved in 100 mL of distilled water. To this solution 0.84 mL of 35 % hydrochloric acid was added. Final pH was adjusted to 0.9. Artificial pancreatic fluid was prepared with 0.25 g of pancreatin and 1.5 g of sodium hydrogen carbonate in 100 mL of water (pH = 8.9). Bile fluid was composed of 0.4 g of bile acid salts dissolved in 100 mL of phosphate buffer. Incubation of probiotics with food or beverage was performed at 37 °C for 20 min in stomach fluid and for 2 hours in intestinal fluids.

2.5 Viability of mixture of probiotic cells in different types of foods and beverages with/without addition of prebiotics

Hydrated probiotic strains from the commercial capsule were solubilized in sterile distilled water were mixed with food matrix and with or without the addition of a prebiotics. Recommended dose of probiotics (1 capsule) was contained in 300 ml of homogenized food or beverage. This prepared mixture was then incubated at room temperature for 20 minutes and then samples were taken for analysis of number of viable cells and/or to perform model digestion. Viability of probiotics cells was determined using cultivation techniques. Determining of the number of cells in cultivation assay was performed after 48 hour of cultivation. As a prebiotic inulin (Sigma Aldrich, Germany) was used.

2.6. Viability of monocultures and mixed probiotic cultures in real foods and beverages in conditions of model digestion

Probiotic bacterial cultures of *Lactobacillus acidophilus*, *Bifidobacterium breve*, or a commercial mixture of probiotic cells (content of capsule Biopron 9) were added to the model or real food/beverages in

the amount of 1.10^{10} CFU. Each of tested probiotics sample was first mixed with food/beverage and incubated for 20 minutes. Then, the samples were exposed to model digestion according to paragraph 2.5. In regular intervals (after or during incubation), the amount of living cells was measured. The viability of probiotics during simulated gastrointestinal conditions was performed using cultivation methods and flow cytometry. The evaluation of viability, CFU number and, thus, influence of individual model and real foods is complicated by continuous growth of probiotic cells in any environment. Thus, the viability was evaluated as a percentage of CFU after incubation in each environment to CFU at the beginning of experiment (= after 20 min incubation in model or real food/beverage).

2.7 Determination of cell viability by cultivation method and flow cytometry

The viability of probiotic bacteria was tested by cultivation assay on Petri dishes with MRS medium by overflow method. After 48 hours of cultivation colony forming units, e.g. bacterial colonies (CFU/mL) were counted.

The viability of bacteria was followed by flow cytometry (Apogee Flow Systems, Hemel Hempstead, UK). As a fluorescent probe Propidium iodide (Sigma-Aldrich, Germany) was used.

3. Results

3.1. Viability of probiotics in model food matrices

The aim of present study was to evaluate viability of tested probiotic cultures in the presence of selected model and real foods and beverages in model conditions of digestive tract. As a model food, solutions with various concentrations of alcohol, sugar, salt, protein and different pH were prepared. Real foods were selected according to most common dietary preferences in population.

3.1.1. Growth of mixed probiotic culture in model foods exposed to artificial digestion

In the first part of this work the influence of model food on concentration and viability of probiotic cells during artificial digestion was studied. The measurement was performed using a flow cytometry and as model foods water, water of pH 3, glucose, protein, sodium chloride and ethanol at different concentrations (see **Table 2**) were used. First, model digestion of the probiotic mixture without addition of food matrix was measured. Based the obtained results, significant decrease of the number of living cells can be observed during artificial digestion. After model digestion of probiotics without the presence of food only 22% of original number of cells was recorded (**Figure 1A**). This experiment simulated a model swallowing an intake of dry probiotic capsule without addition of any food or beverage. On the other hand, after model digestion of probiotics in the presence of water, 731 % of original number of cells was recorded. It is model swallowing intake of a probiotic capsule together with 300 ml of water. Subsequently, the effect of protein (**Figure 1C**) on cell growth during digestion was tested. Two hours after addition of intestinal juices, a significant increase of cell concentration in sample with addition of 10 g/L of bovine albumin was observed. In this sample, the percentage 354 % of the original number of cells was determined.

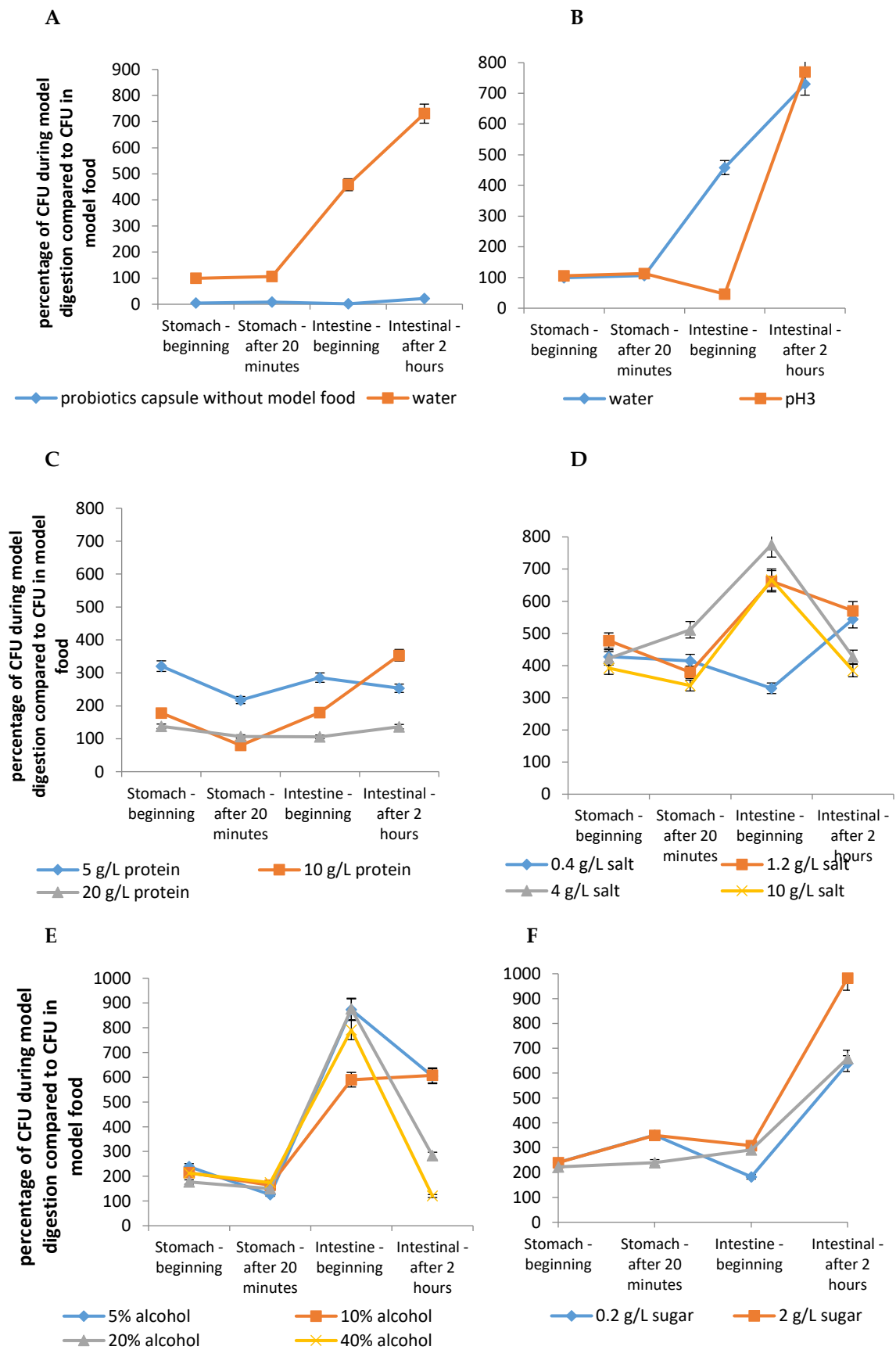


Figure 1. The process of digestion of model foods containing probiotics (commercial probiotic mixture) – A) effect of the presence of food , B) effect of pH, C) effect of proteins; D) effect of salts; E) effect of alcohol; F) effect of carbohydrates.

The effect of salt environment on cell growth during digestion is introduced in **Figure 1D**. After moderate increase of CFU in the environment of intestinal fluids, two hours after addition some decrease of cell concentration was observed (except the lowest concentration used). Moreover, with increasing salt concentration the decrease of cell concentration was more pronounced. For 0.4 g/L NaCl concentration, final CFU value after two hours of model digestion was 544 % of the original CFU value found at the beginning of exposition to artificial digestion.

Next, the effect of alcohol (**Figure 1E**) on cell growth during digestion was monitored. After the addition of intestinal juices, moderate increase of CFU was detected, but after a longer digestion a decrease of cell concentration was observed again. The decrease in cell number with increased alcohol concentration was more pronounced.

Further, the effect of model food with glucose on cell growth during digestion was monitored (**Figure 1F**). The highest increase of number of cells was recorded in sample with 2 g/L of glucose. After two hours of model digestion, 983 % of the original number of cells (about 10x higher CFU) in this sample was determined. Finally, the number of cells in acidic model food was tested (**Figure 1B**). At the beginning of the experiment, a decrease of number of cells in acidic food was observed. However, after two hours of exposition to the intestinal juice, the CFU number for acidic food were already comparable to the water as the model food of a neutral pH.

3.1.2. Viability of probiotic monocultures in model foods exposed to model digestion

In the second part of this work, the influence of model foods on the CFU number and viability of probiotic monocultures of *Lactobacillus acidophilus* and *Bifidobacterium breve* after model digestion in comparison with probiotic mixture was studied (**Figure 2**). After artificial digestion in stomach juice followed by incubation in intestinal juices, CFU number was determined in monocultures and probiotic mixture. The culture of *Lactobacillus acidophilus* exhibited at the end of incubation a high increase of CFU predominantly in foods containing protein, but slightly also in other model foods (**Figure 2A**). Conversely, the strain *Bifidobacterium breve* grew intensively predominantly in model foods containing saccharide (**Figure 2B**). In the commercial probiotic mixture, the highest increase of CFU number after the model digestion in the presence of water at pH=7 and in the presence of acidic solution (pH=3) was found. Furthermore, the CFU increase was detected in foods containing protein and 10% alcohol as well (**Figure 2C**). The resistance of probiotic mixture against acidic pH is probably caused by addition of some protective agent to the preparative, that is intended to prevent damage of cells by acidic stomach environment, as described by the producer.

Similar study described as a suitable food environment for protection of lactic acid bacteria a neutral or slightly acidic pH (5–6). This pH was optimal for growth of probiotics during food manufacturing and cell survival during storage and also during digestion. Water activity can also improve cells protection during digestion, as well as solids and gels. Presence of highly fermentable sugars and fiber promoted survival cells during food storage and digestion too. High fat content exhibited positive effects on cells mainly due to good buffering capacity [19].

3.2 Influence of real foods and beverages on viability of probiotics

3.2.1. Growth of mixed probiotic culture in presence of some real foods and beverages

Influence of different type of foods/beverages and addition of prebiotics on the viability of probiotic cells was observed in following part of this study (**Figure 3**). As a prebiotic in this study, inulin was

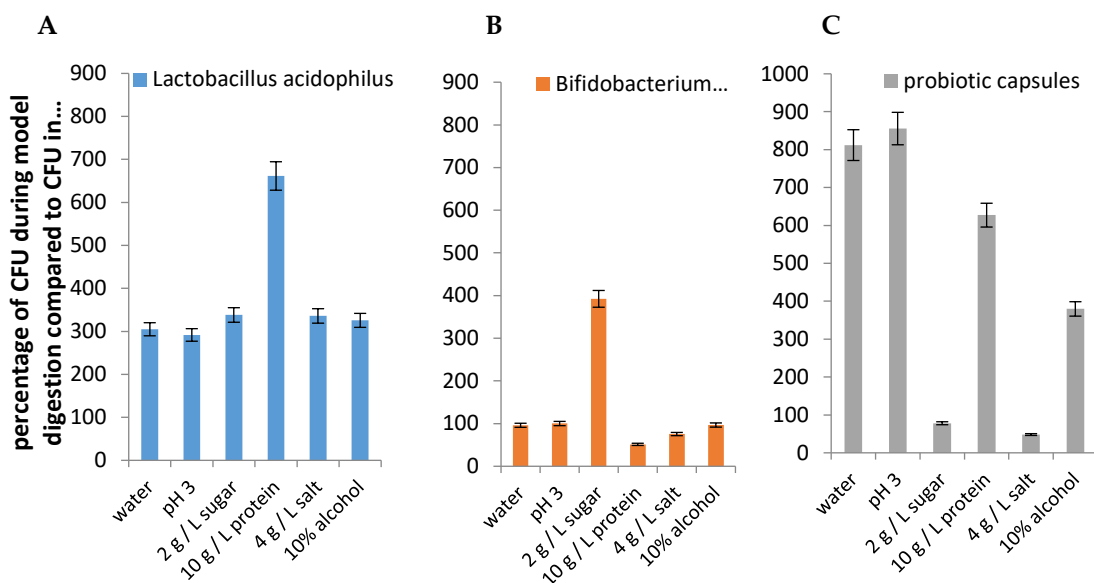


Figure 2. The influence of model foods and the type of probiotic on the number of viable cells after model digestion - A) *Lactobacillus acidophilus*; B) *Bifidobacterium breve*; C) Probiotics mixture

used as one of the most studied and widely used prebiotics [10]. Mixed probiotic culture from capsule was incubated for 20 min in presence of individual foods with and without addition of inulin. As supposed, the highest number of survived probiotic cells was recorded in presence of milk. Higher growth of probiotic cells compared to growth in distilled water was determined for fruits, yoghurt, fruit juice, black tea, coca-cola and beef broth environment. When the probiotic mixture together with a prebiotic was used, similar effect was observed and the highest cell growth predominantly in presence of milk, beef broth and coca-cola was determined (Figure 3).

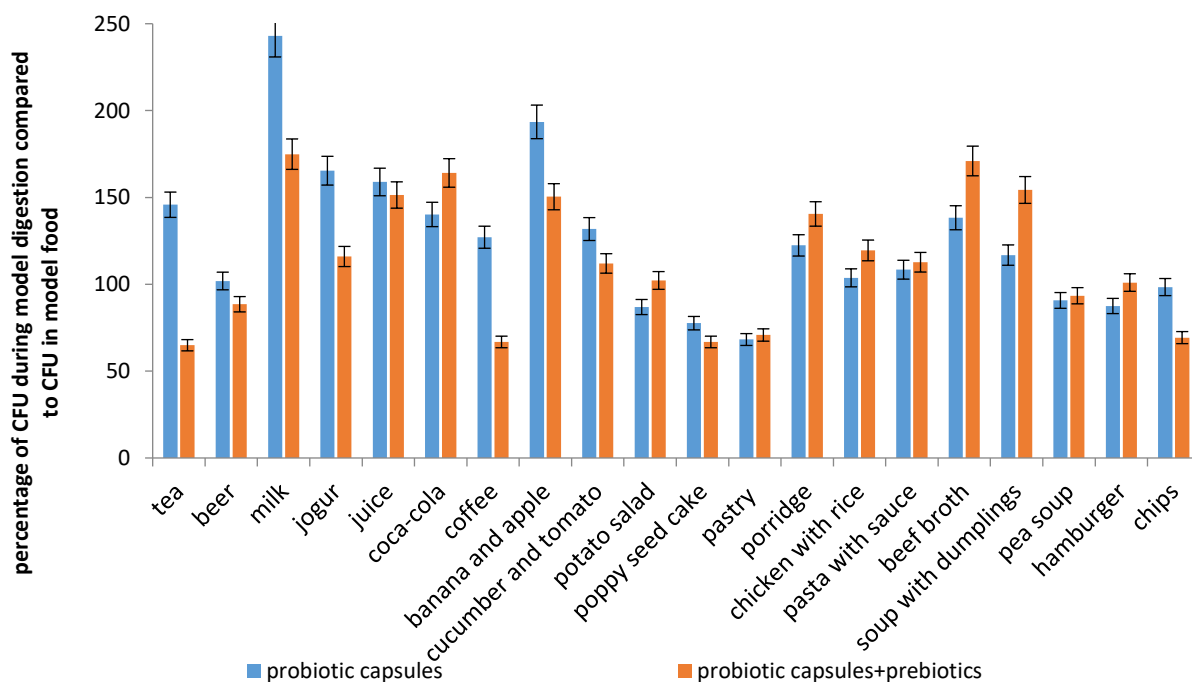


Figure 3. The effect of various foods and the addition of prebiotics on the viability of probiotic cells

3.2.2. Growth of probiotic monocultures and mixed culture in real beverages exposed to model digestion

In the next part of these work was tested the viability and growth of probiotic bacteria *Lactobacillus acidophilus*, *Bifidobacterium breve* and commercial mixture of probiotic strains after passing through the digestive tract with wide range of different type of food and beverages. Tested products can be divided into the following groups: drinks, soups, main courses and snacks (Table 1).

Traditionally, the use of probiotics in dairy beverages has been widely extended. However, since people who are allergic to milk proteins or have severe lactose intolerance cannot consume dairy beverages, non-dairy beverages such as fruits, vegetables and cereals juices may also represent a suitable vehicle to deliver probiotics to consumers, with regard to the stability of the cells during storage [18]. In this study water, black tea, coffee, beer, juice, coca-cola and milk were tested. In all these beverages the highest amount of survived cells and increased growth of probiotic cells was observed in the intestinal environment (Figure 4). These results were also verified by flow cytometry.

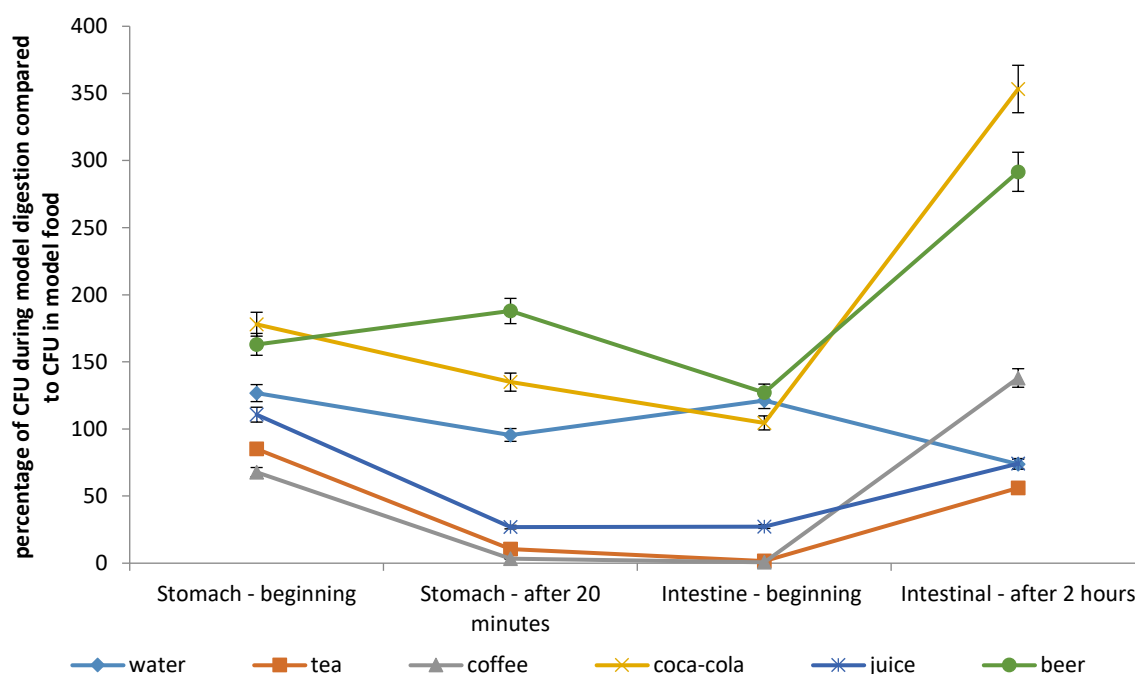


Figure 4. Viability of mixed probiotic culture in selected beverages during the process of model digestion

The influence of real beverages on the CFU number and viability of probiotic monocultures of *Lactobacillus acidophilus* and *Bifidobacterium breve* after model digestion in comparison with probiotic mixture was studied (Figure 5) too. After artificial digestion in stomach juice followed by incubation in intestinal juices, CFU number was determined in monocultures and probiotic mixture. When the monocultures of *Lactobacillus acidophilus* or *Bifidobacterium breve* was used, significant increase (about 2.5 – 3x) of surviving cells was observed only in milk environment (Figure 5A, 5B). This finding confirms the fact, that milk is well-known suitable environment for probiotic bacteria. The probiotic cells from commercial preparation (Figure 5C) showed the highest increase of living cells in a coca-cola and milk environment. In both cases the amount of probiotics cells after passing through the digestive tract was 316 % compare to amount of cells in food before digestion. Interesting was also the finding that increased CFU numbers of probiotic cells after passing through artificial digestion

have also been observed in presence of coffee (269 %), beer (197 %) and black tea (136 %). The mixture of probiotic strains is probably more stable in different environments when compared with monoculture.

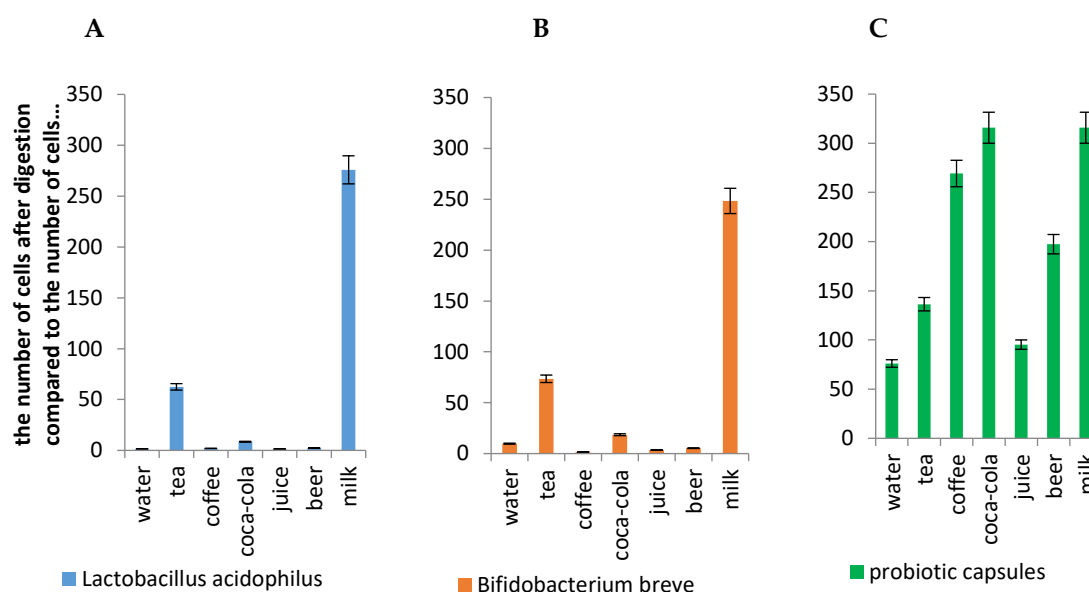


Figure 5. The influence of different types beverages and the type of probiotic on the number of viable cells after model digestion - A) *Lactobacillus acidophilus*; B) *Bifidobacterium breve*; C) Probiotic mixture

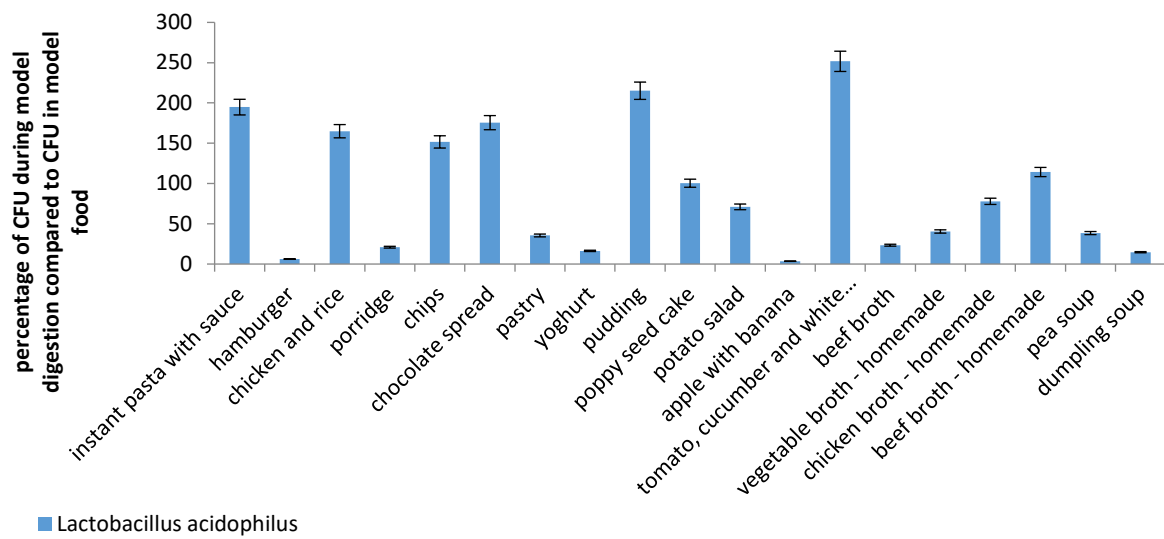
3.2.3. Growth of probiotic monocultures and mixture in some real liquid and solid meals during model digestion

Real foods were divided into liquid meals – soups, solid meals and snacks (see **Table 1**). Influence of all these environments on viability of monocultures of *L. acidophilus* and *B. breve* as well as on viability of probiotics in mixed culture was studied (**Figure 6**).

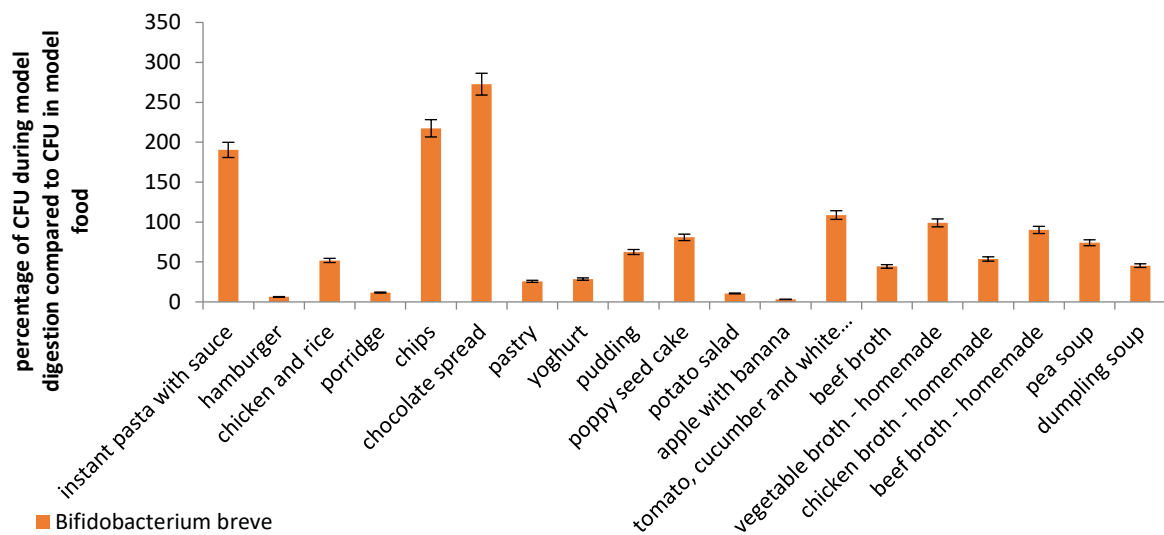
Regarding tested soups, the highest percentage of viable probiotic cells in commercial mixed culture was found in homemade beef broth. The number of probiotic cells after passing through the digestive tract was 335 %, when compared to amount of CFU in the food before digestion. Increased numbers of probiotic cells after passing through the artificial digestion have also been observed in presence of other tested soups. On the other hand, in the case of monocultures, the homemade beef broth was the only environment, in which CFU number of *Lactobacillus acidophilus* slightly increased to 114% after passing through the digestive tract (**Figure 6A**).

The group of tested solid foods was formed by hamburger, instant pasta with cream sauce and chicken with rice as main courses (**Figure 6**). The highest number of survived cells of mixed probiotic culture (**Figure 6C**) was measured after artificial digestion in presence of instant pasta (417 %). Monocultures of *Lactobacillus acidophilus* (**Figure 6A**) and *Bifidobacterium breve* (**Figure 6B**) exhibited a highest increase of survived CFU in presence of instant pasta too. Increased CFU numbers of probiotic cells after passing through the artificial digestion have also been observed in the presence of chicken with rice.

A



B



C

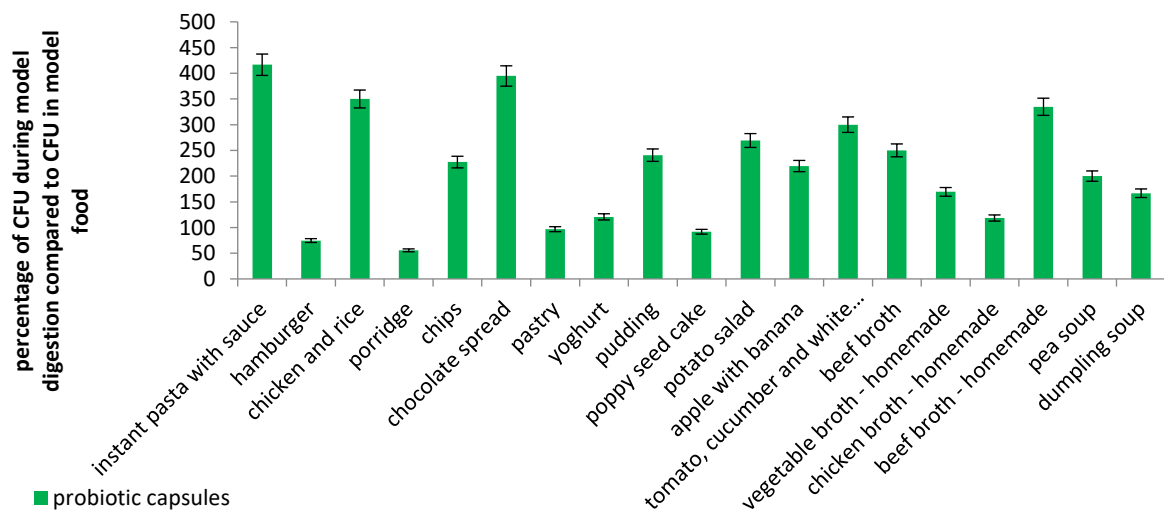


Figure 6. The influence of different types of food and the type of probiotic on the number of viable cells after model digestion - A) *Lactobacillus acidophilus*; B) *Bifidobacterium breve*; C) Probiotic capsule

In the group of snacks (**Figure 6C**), the highest CFU number in mixed probiotic culture exposed to the model digestion was observed in presence of chocolate spread, where CFU number probiotic cells after passing through the artificial digestive tract was 395 %, when compared to CFU in the same food before digestion. Increased CFU number in probiotic mixture was also observed in the presence of mixed vegetables, potato salad, chocolate pudding with whipped cream, salted chips, fruits and yoghurt (**Figure 6C**).

When the monocultures of *Lactobacillus acidophilus* were used, the significant increase of living cells after passing through the artificial digestive tract was observed in presence of mixed vegetables (252 %), chocolate pudding with whipped cream (215 %), chocolate spread (176 %) and salted chips (152 %). Regarding monocultures of *Bifidobacterium breve* similar increase of CFU number was observed mainly in presence of chocolate spread (273%) and salted chips (217%). Small increase of CFU number of probiotics cells was observed in presence of mixed vegetables as well (109 %). Overall, similarly to beverages, mixed probiotic culture was more stable in all tested food compared with monocultures (**Figure 6A-C**).

4. Discussion

Nowadays, probiotics belong to the most popular food supplements. They can be taken in different ways and forms, can be a part of different dietary regimes. For stability and viability of probiotics not only the type of probiotic preparative and processing, but also the foods and beverages ingested simultaneously with probiotics can substantially influence the final effect of probiotics. Moreover, differences in the concentration and viability of probiotic cells when passing through the gastrointestinal tract may be observed. The present work deals mainly with the influence of different types of model and real foods/beverages on the viability of probiotic bacteria during digestion. The aim is to select foods that can increase cell viability during artificial digestion, which may further contribute to the positive effect of probiotic cells on human health.

Species of *Lactobacillus* and *Bifidobacterium* are the most commercial probiotics available in the foods market [18]. Therefore, in this work the influence of food and beverages was tested on monocultures of *Lactobacillus acidophilus*, *Bifidobacterium breve* and for comparison also on commercial mixture of probiotics containing 9 different probiotic strains.

To reach probiotic status and ability to promote health benefits, it is necessary to evaluate cells resistance to the digestion process [10]. In this study, probiotic cultures were shortly incubated in selected foods and beverages and then the viability of probiotics was tested in model artificial digestive tract. The conception was proposed to find suitable and, conversely, completely inappropriate food taken into the body together with probiotics. First, incubation of probiotics in the environment of various model foods was performed and followed by artificial digestion. As a model food, solutions with various concentrations of alcohol, sugar, salt, proteins and acid were prepared.

In further part, real foods and beverages were tested in similar way. Recently it was found that some of food matrices are during storage and simulated gastrointestinal conditions more protective than others [4,10,18]. In particular, special attention has been paid to dairy products such as cheese, yogurt, and fermented milk. Cheese has a potentially good matrix for delivery of probiotic due to several characteristics including its higher pH value, greater buffering capacity, greater fat content and nutrient availability, and lower oxygen content. Also fruits and vegetables have been found to be an ideal addition to probiotic foods, likely because they provide essential nutrients for bacterial growth

[4]. As well as fiber-rich products such as fruit and grain can increase viability of probiotic bacteria during storage and simulated gastrointestinal conditions [18,19].

The tolerance of probiotic bacteria to gastric and small intestine conditions seems to be significantly influenced by the food carrier. Many studies showed that vegetable matrices could improve probiotics vitality during the gastric transit. It was confirmed for example the good protection of probiotics against simulated gastric juices by carrot juice same like at by dairy matrices [2]. In other studies the food matrix impact on lactic acid bacteria viability during food digestion of dairy versus non-dairy products [18]. When milk versus fruit juices were compared, the high tolerance for bile and pepsin in milk environment was observed. When comparing different flavours of juice, banana and carrot juice exhibited highly positive effect when compared with orange juice [4]. Very good positive effects on probiotics compared dairy product have also been reported for pasta [7]. When comparing different dairy products, the best results were obtained with cheese, milk and yoghurt, and finally ice cream [13]. However, for different strains, differences in the order of these matrices were recorded [19].

The purpose of this work was to try to find food matrices that will provide optimal conditions for growth of probiotics and possible protection against the effects of the digestive system. This study contains a set of original data represented by results of incubation of probiotics in presence of many different types of complex food matrixes and exposed to model digestion. Meals containing combination of plant and animal foods such as meat, pasta, cream and some of the most popular beverages were tested. Some of our data agree with previously published studies focused on influence of fruit and dairy products [4,7,18-19].

Based on the results it can be concluded that the best way appears to be the combination of mixtures of probiotic with instant pasta with cream sauce, chocolate spread or homemade beef broth. As the best option among beverages seems to be milk. Nevertheless, other beverages such as tea, coffee, coca-cola and beer can be recommended. Further, we can conclude that probiotic microorganisms have survived better in presence of meals, when compared with consumption with beverages only. Higher growth of probiotic cells was observed in foods containing high concentration of sugar and fat or their optimal combination. These data are confirmed also by recent study, where are carbohydrates the most widely used protective compounds during dehydration, storage and exposure of probiotic to gastrointestinal tract [16].

5. Conclusions

The present work dealt with the influence of different types of model and real foods on cell viability during digestion. The main goal of the work was to select a suitable food that demonstrates an increase in cell viability during digestion, which can further lead to an increase in the positive effect of probiotic cells on human health.

As model foods still water, acidified water (pH=3), and various concentrations of glucose, proteins, sodium chloride and ethanol were used. The CFU number and viability of probiotic monocultures of *Lactobacillus acidophilus* and *Bifidobacterium breve* during model digestion were monitored and compared with commercial probiotic mixture. The highest growth of *Lactobacillus acidophilus* cells was detected in foods containing protein. On the contrary, *Bifidobacterium breve* exhibited the best growth in presence of foods containing saccharide. The best growth of mixed probiotic culture was

determined in water at neutral pH and in acidic environment, as well as in the presence of foods containing protein and 10% alcohol.

Regarding real foods and beverages, the best environment food for increase of probiotic viability was pasta with cream sauce, chocolate spread and homemade beef broth. The high CFU numbers of probiotic cells after passing through the artificial gastrointestinal tract have also been observed in presence of chicken with rice, mixed vegetables, potato salad, chocolate pudding with whipped cream, salted chips, fruits and yoghurt. Among beverages, the best option was milk, but acceptable for viability of probiotics were coca-cola, coffee, beer and black tea.

It can be concluded that probiotics are more viable when consumed with meals compared to the beverages only. Viability of mixed probiotic culture was higher in all environments when compared with both monocultures.

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