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Lactic Acid Bacteria and Fermentation of Cereals and Pseudocereals

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Abstract

The usage of lactic acid bacteria (LAB) in food as starters in fermentation technologies has a long tradition. Although the theorized idea of host-friendly bacteria found in yoghurt has been formulated only over a century ago, both groups are widely used nowadays. Lactic acid bacteria alone or with special adjunct probiotic strains are inevitable for the preparation of various specific fermented and probiotic foods. Moreover, because of their growth and metabolism, the final products are preserved for a certain time. Growth dynamics of probiotic LAB and Fresco DVS 1010 in milk- and water-based maize mashes with sucrose or flavours (chocolate, caramel and vanilla) were evaluated in this study. Although milk is typical growth medium for the LAB growth, observed strains showed sufficient growth in each of prepared mashes as well as they were able to maintain their content above 10^6 CFU ml⁻¹ during storage period (6°C/21 d). Designed flavoured mashes were acceptable from the microbiological point of view, but according to the sensory evaluation they were provided with an attractive overall acceptability and are adequate alternative for celiac patients, people suffering from milk protein allergies or lactose intolerance.

Keywords: lactic acid bacteria, fermentation, biopreservation, probiotics, functional products

1. Introduction

For centuries, human civilization had used different approaches to preserve different types of food products. If we look back in history, we can find the preparation of different types of foods, for example, alcoholic beverages by ancient Egyptians, the preparation of yoghurt and kefir by

the nomadic people from central Asia, fermentation of meat by the Germanic tribes and fish by the Eskimos, preparation of boza by the ancient Persians or fermenting maize by the native tribes in pre-Columbian America [1]. The earliest records about fermentation process were dated back to 6000 BC, and thus fermentation represents one of the oldest food preservation methods [2, 3]. The ancient people probably did not have any knowledge of microbiology, but in the middle of the nineteenth century, Louis Pasteur significantly contributed to the understanding of the fermentation process itself. He established the role of microorganisms and proved that there are many different kinds of fermentation [3]. The original and primary purpose of fermentation was a preservation effect. Subsequently, with the development of many available preservation technologies, plenty of fermented foods were therefore manufactured because of their unique flavours, aromas and textures much appreciated to a consumer [4, 5]. Fermentation process created plenty of traditional food products, such as milk products (cheese, butter and yoghurt), fermented meat, plants and fruits (sausages, silage, sauerkraut, olives and grapes) and finally fermented cereal products such as bread and beer [6]. Fermented food and beverages are defined as those that have been subjected to the effect of microbial enzymes, particularly amylases, proteases and lipases that cause biochemical transformation of polysaccharides, proteins and lipids to non-toxic variety of desirable products with tastes, aromas and textures attractive to a consumer [4, 7].

In food fermentations, conditions of treatment and storage create an environment in which certain types of organism can flourish and these have a benign effect on the food rather than spoiling it. The majority of fermented foods is produced by the activity of lactic acid bacteria (LAB) and fungi, principally yeasts but also, to a lesser extent, moulds. Both groups of organisms share a common ecological niche, are able to grow under conditions of low pH and reduced water activity, although only lactic acid bacteria and facultative yeasts will prosper under anaerobic conditions. They frequently occur together in fermented products, dairy and non-dairy, but in some cases, they play the role of a spoilage agent [8].

Microorganisms responsible for the fermentation process may be presented naturally in the substrate, or may be added as a starter and adjunct cultures [9].

2. Lactic acid bacteria

Lactic acid bacteria (LAB) represent an ubiquitous and heterogeneous species with common feature of lactic acid production as a result of sugar metabolism which leads to an acidification of the environment down to a pH of 3.5 [10]. The monograph by Orla-Jensen (1919) formed the basis of the present classification of LAB that take into account the cellular morphology, mode of glucose fermentation, growth temperature and sugar utilization possibilities [11]. Taxonomically, LAB are divided into two distinct phyla: *Firmicutes* and *Actinobacteria*. Within the *Firmicutes* phylum genera such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Tetragenococcus*, *Aerococcus*, *Carnobacterium*, *Weissella*, *Alloiooccus*, *Symbiobacterium* and *Vagococcus* belong. Within the *Actinobacteria* phylum, lactic acid bacteria belong to the *Atopobium* and *Bifidobacterium* genera [12].

Lactic acid bacteria are Gram-positive, non-sporulating, non-pigmented and non-motile rods and cocci, most of which are non-respiring but aerotolerant anaerobes. They lack cytochromes and porphyrins and are therefore catalase- and oxidase-negative. LAB tend to be nutritionally fastidious, often requiring specific amino acids, B-vitamins and other growth factors. Some do take up oxygen through the mediation of flavoprotein oxidases, thus producing hydrogen peroxide and/or re-oxidizing NADH during dehydrogenation of sugars. The cellular energy is derived from the fermentation of carbohydrates to produce major lactic acid. They use one of two different pathways and this provides a useful diagnostic feature in their classification. Since many species of lactic acid bacteria (LAB) and other food-associated bacteria had a long historical association with human foods, they are recognized as generally regarded as safe (GRAS) bacteria. Infections by LAB are characterized as opportunistic that rely on host factors rather than on intrinsic pathogenicity. Only rare cases of clinical infections have been reported in humans, for example, in patients with endocarditis or with immune deficiency [8, 12–15].

Homofermentative organisms produce only lactic acid from the glucose fermentation during the Embden-Meyerhof-Parnas glycolytic pathway. Heterofermenters produce roughly equimolar concentration of lactate, ethanol/acetate and carbon dioxide from glucose (**Table 1**).

Genera	Morphology	Fermentation	Lactate isomer	DNA (mole % G-C)
<i>Lactococcus</i>	Cocci	Homo	L	33–37
<i>Lactobacillus</i>	Rods	Homo/hetero	D, L, DL	32–53
<i>Leuconostoc</i>	Cocci	Hetero	D	38–41
<i>Streptococcus</i>	Cocci	Homo	L	40
<i>Pediococcus</i>	Cocci	Homo	DL	34–42

Table 1. Principal genera of the lactic acid bacteria [8].

2.1. Starters used in lactic acid fermentation

Genera *Lactobacillus* is recognized as being phylogenetically very heterogeneous and this is evidenced from broad interval of % G-C content. They are in general characterized as Gram-positive, microaerophilic, non-spore-forming and non-flagellated rods or coccobacilli. They are commonly found in a diversity of environments, in dairy and meat-fermented products, in fermented and pickled vegetables, adhered on human-mucosal surfaces (in the gastrointestinal and vaginal tract) as well as in soil and plants [16, 17]. Intestinal lactobacilli (*Lb. rhamnosus*, *Lb. acidophilus*, *Lb. reuteri*, *Lb. plantarum* and *Lb. paracasei*) interact with the host and have been linked with numerous health benefits [18–20]. *Lb. reuteri* is one of the most probiotic bacteria, which are added to infant dried milk formula for babies with lactose intolerance or for realimentation after diarrhoea [21]. Lactobacilli are naturally presented in breast milk, especially species of *Lb. fermentum*, *Lb. rhamnosus*, *Lb. gasseri* and *Lb. salivarius* [18, 22–25]. Liptáková and co-workers [26] identified frequently *Lb. plantarum* from breast milk of healthy mothers.

Lactobacillus species are divided into three groups based on fermentation end products: obligate homofermenters, facultative heterofermenters and obligate heterofermenters [17, 27]. Obligate homofermenters ferment hexoses almost exclusively to lactate but are unable to ferment pentoses or gluconate (*Lb. helveticus*, *Lb. acidophilus*, *Lb. delbrueckii* and others). *Lb. acidophilus* strains are the best known of the health-promoting lactobacilli and it is a part of human gut microflora. As probiotic strain is added to dairy foods for its physiological benefits. The facultative heterofermenters ferment hexoses via the EMP pathway and pentoses due to phosphoketolase activity to lactate, acetate, formic acid and ethanol (*Lb. plantarum* and *Lb. casei*). Obligate heterofermenters such as *Lb. brevis*, *Lb. reuteri*, *Lb. fermentum* or *Lb. kefir* use the phosphoketolase pathway for hexoses and pentoses fermentation and the main products of fermentation are lactic and acetic acid (or ethanol), and carbon dioxide [13, 15, 28].

Genera *Lactococcus* contains the major mesophilic microorganisms used for lactic acid production especially in dairy fermentations (sour milk and cream, lactic butter, fresh, soft and hard cheeses of artisanal and commercial origin). Some of them are suitable for cereal and pseudocereal fermentations [29, 30]. Joseph Lister made the first reported isolation of microorganism responsible for milk fermentation in 1873. He named the culture *Bacterium lactis* that was changed to *S. lactis* later. Orla-Jensen in 1919 differentiated mesophilic lactic streptococci into *S. lactis* and *S. cremoris*, which were included in Group N *Streptococci* [29]. On the present, the genus *Lactococcus* comprises nine species: *L. lactis* (including the subspecies *lactis*, *cremoris* and *hordniae*), *L. garvieae*, *L. piscium*, *L. plantarum*, *L. raffinolactis*, *L. chungangensis*, *L. fujiensis*, *L. formosensis* and *L. taiwanensis* [31, 32]. *L. cremoris* is unable to ferment maltose and ribose, to grow at 4% of salt and to hydrolyse arginine in comparison with *L. lactis*. *L. lactis* subsp. *lactis* var. *diacetyllactis* converts citrate to diacetyl, carbon dioxide and acetone responsible for a creamy and buttery aroma in fermented milks, cream and butter and in Camembert, Emmental and Cheddar type of cheeses [13, 15, 33].

Many strains of *L. lactis* produce bacteriocins, which have antimicrobial activity especially against a narrow spectrum of *Lactococci*; however, nisin and lacticin 3147 have much broader activity against a wider range of Gram-positive bacteria. Nisin has been accepted as a food additive to control contaminating microbiota [11, 29]. Sadiq et al. [34] isolated three bacteriocinogenic strains *L. lactis* described as TI-4, CE-2 and PI-2 that were effective against *B. subtilis* and *S. aureus* and the maximum of bacteriocins (nisin A and nisin Z) were produced at 25 and 30°C and at pH 5 and 8, respectively.

Leuconostocs (predominantly *Ln. mesenteroides* subsp. *cremoris*) are the most commonly used heterofermentative dairy lactic acid bacteria that are flavour-producers in a number of fermented dairy products and cheeses. The fermentation of citrate is important in diacetyl and carbon dioxide formation in some types of cheeses. The genus *Leuconostoc* consists of 12 species isolated from plant, fermented foods (meats and vegetables or dairy products), vacuum-packaged, cold-stored meat, honey, Ethiopian coffee fermentation, kimchi, palm wine, cane juice and human clinical sources: *Ln. mesenteroides*, *Ln. pseudomesenteroides*, *Ln. carnosum*, *Ln. citreum*, *Ln. fallax*, *Ln. gasicomitatum*, *Ln. gelidum*, *Ln. holzapfelii*, *Ln. inhae*, *Ln. kimchii*, *Ln. lactis*, *Ln. palmae* [35–38].

Twelve species of genera *Pediococcus* are currently recognized: *P. acidilactici*, *P. pentosaceus*, *P. argentinicus*, *P. cellicola*, *P. clausenii*, *P. damnosus*, *P. ethanolidurans*, *P. inopinatus*, *P. lolii*, *P. parvulus*, *P. siamensis* and *P. stilesii*. In contrast to other cocci in the LAB, pediococci usually do not form chains of cells [35, 39]. Pediococci are associated with dairy products and dairy environment and have potential impact on texture due to exopolysaccharides production. Garai-Ibabe et al. isolated two strains of *P. parvulus* (CUPV1 and CUPV22) that enable to produce high concentration of 2-substituted (1,3)- β -D-glucan increasing viscosity of the growth media [40]. Pediococci are often found in a large number of several fermented meat and fish products, fermented beans, cereals, olives or sauerkraut. Some strains are proposed to have probiotic activity due to their ability to survive and adhere to the gastrointestinal tract and due to reported immune modulation capability [13, 39, 41].

Streptococcus derives from the Greek 'streptos' –easily twisted like a chain– and 'kokkos' – grain/seed and the term was firstly used in 1874 by Billroth as a descriptor for the chain-forming, coccoid-shaped bacteria. Rosenbach (1884) firstly applied the generic name *Streptococcus* when describing *S. pyogenes*, the chain-forming coccus isolated from suppurative abscess in human. In 1906, Andrewes and Horder examined 1200 streptococci isolated from human, air and milk sources, and on the basis of sugar metabolism, reduction of neutral red and growth characteristics in milk, they distinguished eight groups. Sherman in 1937 produced the first comprehensive systematic classification of streptococcal isolates from environmental, commensal and hospital sources. He excluded from the genus *Streptococcus* all strictly anaerobic cocci and pneumococci because of their extreme sensitivity to bile and introduced four primary divisions: pyogenic, enterococcus, lactic and viridans group [42]. The results of molecular taxonomic studies allowed the major changes in the classification of *Streptococcus* spp.: the 'lactic' streptococci now constitute the genus *Lactococcus* and some members from Sherman's 'enterococcus' division became foundation members of the genus *Enterococcus* [43]. The subdivision of *Streptococci* into seven groups is based on 16S rRNA gene sequence data correlated well with the results of DNA-DNA re-association experiments and numerical taxonomic studies [44–46].

The only *Streptococcus* sp. useful in milk fermentation (production of yoghurt and Swiss- or Italian-type cooked cheeses such as Grana Padano, Gorgonzola, Mozzarella or Fontina) is *S. thermophilus* var. *salivarius*. It has the status of GRAS in the USA and a Qualified Presumption of Safety in European Union due to its long history of safe use in food manufacture. The end products of lactose fermentation are lactate, acetaldehyde and diacetyl. Some strains are able to produce thermophilins, proteinaceous compounds that are inhibitory against listeria and clostridia, especially thermophilin 13 and 1277 have a broad inhibitory spectrum [15, 27, 28, 47–50].

Species from the genus *Bifidobacterium* were originally identified from stool samples of breast-fed infants as bacteria with a strange and characteristic Y shape in 1899 by Tissier and named *B. bifidus*. In 1924, Orla-Jensen recognized the existence of the genus *Bifidobacterium* as a separate taxon but due to the similarities of bifidobacteria with the genus *Lactobacillus* they were included in this genus. In 1957, Dehnart realized the existence of multiple biotypes of

Bifidobacterium and proposed a scheme for the differentiation of these bacteria based on their hexose fermentation pathway [51].

The most frequently found strains in the human gastrointestinal tract include *B. adolescentis*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. pseudocatenulatum*, *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, *B. dentium* and *B. angulatum* [52]. Bifidobacteria represent up to 25% of the cultivation faecal microbiota in adults and 80% in infants [53]. According to Matsuki and co-workers [54], the most often isolated bifidobacteria from adult intestinal tract are *B. catenulatum*, *B. longum* and *B. adolescentis*, whereas *B. breve*, *B. infantis* and *B. longum* predominate in the infant intestine.

The most important species of *Bifidobacterium* for probiotic application are *B. longum*, *B. bifidum* and *B. animalis*. Children receiving *Bifidobacterium*-supplemented milk-based formula (*B. lactis* Bb-12 strain) were protected against symptomatic rotavirus infection. Daily consumption of three cups/day of *B. longum* yoghurts decreased erythromycin-associated gastrointestinal disorders. *B. bifidum* NCFB 1454 was found to be active against certain species of *Listeria*, *Bacillus*, *Enterococcus*, *Pediococcus* and *Leuconostoc* due to bifidocin B production [15, 16, 28, 51, 53, 55, 56].

3. Antimicrobial compounds produced by lactic acid bacteria

Lactic acid bacteria may produce substances and thus create conditions harmful for undesired bacteria, yeasts and moulds which lead to the increase of food shelf life [57]. Temperature and incubation period are the main factors modulating production of antimicrobial substances. Sathe et al. [58] in their study evaluated the impact of the growth phase on antimicrobial activity of *Lb. plantarum* at 30°C. The evaluated strain showed maximal antimicrobial activity at the end of exponential phase of growth, and in stationary phase after 48 h of cultivation, decline in antimicrobial activity was observed. These results are consistent with the study of Batish et al. [59] who observed maximal antimicrobial activity of the same strain after 48 h of incubation at 30°C. The main product of fermentation by lactic acid bacteria is mostly lactic acid. However, under aerobic conditions, carbon dioxide and acetic acid are created as a result of oxidative dissimulation, while hydrogen peroxide as an intermediate product is formed [27]. Most of the isolated and identified antimicrobial substances produced by lactic acid bacteria are with low-molecular weight composed of organic acids, reuterin, hydrogen peroxide, hydroxyl fatty acids, phenolic and proteinaceous compounds [60].

When lactic acid is produced, the pH decreases and consequently the organic acids or small fatty acids (SFAs) become undissociated and represent the main antimicrobial activity of the LAB [61]. It has been shown that organic acids penetrate bacterial membrane of the target microorganism and inhibit transport mechanism in the cell by reducing pH values [62]. The effect of acids depends not only in combination with lowering pH and reduction of redox potential but also on the type and concentration of acid presented in the environment [63]. Acetic acid in comparison to lactic acid was described as being more effective, and is able to inhibit growth of moulds, yeasts and bacteria [5]. Propionic acid inhibits moulds and selected

Gram-positive microorganism [62]. Phenyllactic acid and pyroglutamic acid are able to inhibit growth of *Aspergillus niger*, *A. flavus* and *Penicillium expansum*, and both were isolated from cell-free extract of *L. plantarum* and *Lb. rhamnosus* GG (LGG) [60, 64]. Liptáková et al. [64] mathematically predicted the inhibitory effect of *Lb. rhamnosus* GG (LGG) on the growth dynamics of *Candida maltosa* YP1 and *Geotrichum candidum* yeasts. At 18°C, growth rates of the yeasts in mixed cultures decreased about 50% compared with rates of its pure cultures. The effectiveness of growth inhibition of *C. maltosa* was dependent on initial LGG concentration; the most antagonistic activity of lactobacilli was determined at log 4 and log 6 initial concentration (**Figure 1**). Greifová et al. [65] described the inhibitory effect of D, L-phenyllactic acid on moulds such as *Alternaria alternata*, *A. flavus*, *Cladosporium herbarum*, *Fusarium nivale*, *Mucor racemosus* and *P. funiculosum*.

Liptáková and co-workers [66] focused on the growth of yoghurt contaminant *C. maltosa* YP1 in milk as influenced with initial different numbers of *Lb. rhamnosus* VT1 (ranged from 1 to 15% v/v) and temperature. The growth parameters of yeast in dependence on the lactobacilli counts at 17°C are summarized in **Table 2**. The antagonistic relationship between *C. maltosa* YP1 and *Lb. rhamnosus* VT1 was based not only on the lactic acid but it was consequence of the other antimicrobial, non-proteinaceous and non-saccharidic substances, identified by Plocková et al. [67] and also pyroglutamic acid, later identified by Liptáková et al. [64].

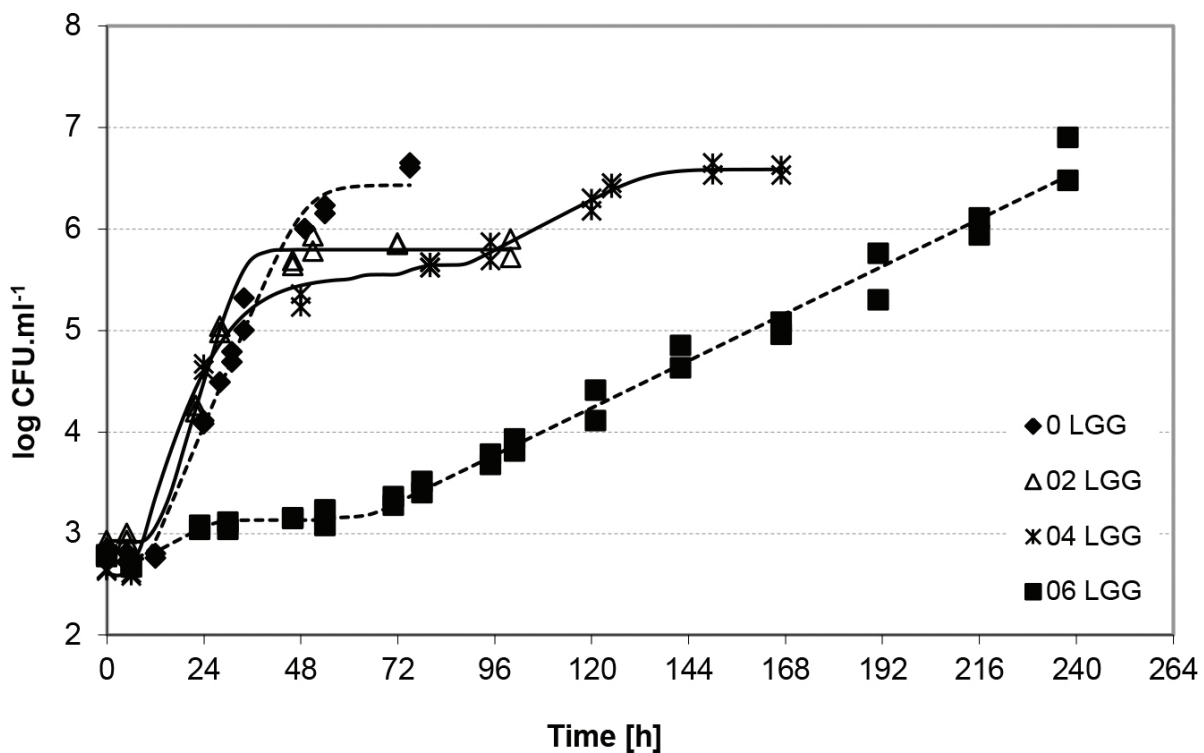


Figure 1. Growth dynamics of *C. maltosa* YP1 in co-culture with *Lb. rhamnosus* GG at 18°C in dependence on various initial lactobacilli concentration (♦ without LGG addition, Δ 2 log LGG initial counts, × 4 log LGG initial counts, ■ 6 LGG initial counts).

Initial inoculation of <i>Lb. rhamnosus</i> VT1 (% v/v)	Growth rate (log CFU ml ⁻¹ h ⁻¹)	Lag-phase duration (h)
1.0	0.062	0.1
	0.064	0.1
5.0	0.055	5.5
	0.052	8.1
10.0	0.046	72.9
	0.046	74.2
15.0	0.041	76.4
	0.043	73.1

Table 2. Values of growth rate (Gr) and lag time (λ) of *C. maltosa* YP1 in milk in dependence of initial numbers of *Lb. rhamnosus* VT1 at $17 \pm 0.5^\circ\text{C}$.

3.1. Hydrogen peroxide

Most lactic acid bacteria produce hydrogen peroxide in the presence of oxygen. After its accumulation, inhibitory effect is mediated through oxidizing effect on membrane lipids and cell proteins of targeted microorganism. The antimicrobial activity of the compound in lower concentrations mostly in food is enhanced by treatment with the formation of hypothiocyanite catalysed by lactoperoxidase system [68]. Fitzsimmons and Berry [69] reported in their study the inhibitory effect of hydrogen peroxide on the growth of *C. albicans*. The minimum inhibitory concentration is less than 0.025% [60].

3.2. Carbon dioxide

Carbon dioxide at low concentrations may stimulate the growth of selected bacteria. Creating an anaerobic environment may be toxic to some aerobic food microorganisms through its action on cell membranes and its ability to reduce internal and external pH values [5].

3.3. Bacteriocins

Bacteriocins are ribosomally synthesized antimicrobial group of heterogeneous peptides with antimicrobial effect that kill or inhibit the growth of other bacterial strains. Typically, LAB bacteriocins have a narrow antibacterial spectrum, but some strains may also produce bacteriocins with a broad antibacterial spectrum. Selected lactic acid bacteria may inhibit the growth of Gram-positive pathogenic and spoilage bacteria, as well as yeasts. It has been reported that bacteriocins also inhibit the growth of some Gram-negative species. Lozo et al. [70] showed the production of bacteriocin 217 (Bac 217) by the strain *Lb. paracasei* subsp. *paracasei* BGBUK2-16 isolated from traditional home cheese that shows inhibitory effect against *Staphylococcus aureus*. Strains of *Lb. fermentum*, *Lb. pentosus*, *Lb. paracasei* and *Lb. rhamnosus* isolated from traditional corn drink made of wheat have produced active bacteriocins against *Escherichia coli*, *P. aeruginosa* and *E. faecalis* [71]. Valdés-Stauber and Scherer [72] isolated and characterized Linocin M18, bacteriocin produced by *B. linens* M18 in stationary growth phase. This bacteriocin was able to inhibit *Listeria* spp., especially *L. monocytogenes*, *L. innocua*, *L.*

ivanovii and several coryneforms, Gram-negative bacteria were insensitive. Corsetti et al. [73] in their study described the antimicrobial substances in sourdough and identified them as a bacteriocins-like inhibitory substance. Some leuconostocs, especially *Ln. mesenteroides* subsp. *mesenteroides* Y105 and UL5, are able to produce bacteriocins with antilisterial activity [37, 74]. Some strains of *Pediococcus* spp. may have antimicrobial effect by the production of pediocins against undesirable and pathogenic microorganisms, for example, *Listeria* spp. and *Clostridium perfringens* [75]. Gurira and Buys [76] isolated *P. acidilactici* and *P. pentosaceus* from Bouquet and Gouda cheeses as non-starter lactic acid bacteria which had inhibitory potential against *L. monocytogenes* ATCC 7644 and *B. cereus* ATCC 1178 through the action of pediocins. Altuntas et al. [77] confirmed the antilisterial effect of pediocin producing strain *P. acidilactici* 13 in their study.

3.3.1. Reuterin

Reuterin is a product of glycerol fermentation produced during stationary phase by *Lb. reuteri*, *Lb. brevis*, *Lb. buchneri*, *Lb. collinoides* and *Lb. coryniformis* under anaerobic conditions, which enables to suppress ribonuclease activity [60]. Reuterin has a wide inhibitory spectrum against Gram-negative and Gram-positive bacteria, yeasts, fungi and protozoa: *Salmonella*, *Shigella*, *Clostridium*, *Staphylococcus*, *Listeria*, *Candida* and *Trypanosoma* [78]. An inhibitory effect on the growth of genus *Aspergillus* and *Fusarium* has been reported. The addition of glycerol to the media containing lactic acid bacteria producing reuterin increased its antifungal activity [60].

4. Probiotics and functional foods

4.1. Probiotics

The word probiotic originated from Greek meaning 'for life'. The first definition of probiotics was described by Vergin, 1954, as the opposite to antibiotics, and 1 year later Kolb proposed that the microbial imbalance in the human body as a result of antibiotic therapy could be restored by probiotics. Parker in 1974 defined the probiotics as organisms and substances that contribute to gut-microbial balance. Most frequently cited definition is that of Fuller's (1992), who defined them as 'a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance'. According to the recommendations of a Food and Agriculture Organization/World Health Organization (FAO/WHO)-working group on probiotics suggested definition describes probiotics as live microorganisms that when administered in adequate amounts confer health benefit on the host (2002). Health benefits must be scientifically established by clinical studies in humans and published in peer-reviewed journals [79]. A number of genera and strains of bacteria (*Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *S. salivarius* subsp. *thermophilus*, *E. faecium*, *E. faecalis*, *E. coli*, *B. cereus*, *B. subtilis*, *B. clausii*, *B. coagulans*, *B. licheniformis* and *B. polyfermenticus*) and yeast *Saccharomyces boulardii* are used as probiotic mostly in dairy products (milks, yoghurts and probiotic cheeses) but also in non-dairy food and beverages such as dry sausages, soy milk

drink, juices, fermented cereal products Boza, Bushera, Mahewu and Pozol [57, 80–85]. Lee and co-workers [86] investigated the probiotic potential of *B. polyfermenticus* KU3 isolated from kimchi. The spore suspension was resistant to artificial gastric juice and survived for 24 h in artificial bile acid, adhered strongly to HT-29 cell line and anti-carcinogenic activity of *B. polyfermenticus* KU3 was observed. Cell *B. polyfermenticus* strongly inhibited the proliferation of various cancer cell lines such as HeLa, LoVo, HT-29 and MCF-7 (percentage of inhibition between 90.5 and 96.9%). Liptáková et al. [87] observed comparable inhibition effect on the proliferation of HeLa and Caco-2 cells due to the adhesion and metabolism of probiotic *Lb. acidophilus* 145 (95–96%) and *Lb. rhamnosus* GG (68%).

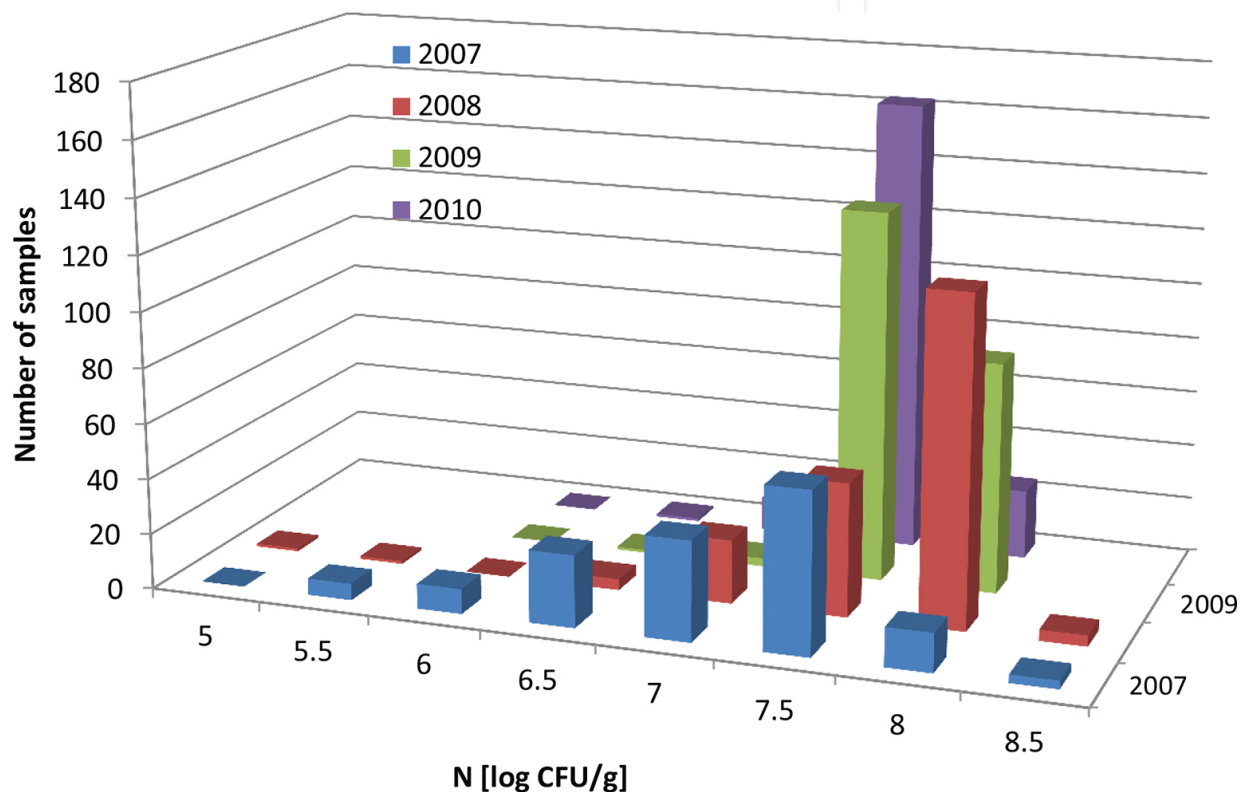


Figure 2. *Lactobacillus acidophilus* contents in the fermented milk at the end of shelf life.

The choice of which microbe to use as a probiotic is determined by many factors: probiotics have to be safe, non-pathogenic and non-toxic species, survive the passage through the intestinal tract and adhere to the intestinal mucosa and organic acid production, lactic and acetic [57, 79]. According to Tripathi and Giri [85], the viability of probiotics in food is affected by many factors such as pH, water activity, redox potential of foods, presence of salt, sugar, hydrogen peroxide, bacteriocins, aroma and colouring compounds, processing, packaging and storage conditions. Probiotic foods should preferably be stored at a temperature between 4 and 5°C. The highest viability of *Lb. acidophilus* LA-5 in yoghurt was observed 20 days at 2°C, but for *B. lactis* BB 12 the optimum storage temperature was 8°C [88, 89]. To realize health benefits on host, probiotic microorganisms must be viable and available in a high concentration of about 10^6 to 10^7 CFU/ml or g at the end of shelf life of product, so minimum therapeutic

daily dose is usually considered as 10^8 to 10^9 CFU/ml or g [16]. Liptáková et al. [90] determined the concentration of *Lb. acidophilus* 145 in acidophilus milk at the end of shelf life during storage at 6, 8 and 10°C . The number of probiotic *Lb. acidophilus* 145 ranged from 6 to 7 log counts. Over a period of 5 years (2007–2011), Valík et al. [91] monitored the contents of *Lb. acidophilus* in the fermented milk at the end of shelf life. The average values in log CFU/g ranged in interval from 6.85 to 7.47, respectively. In the years 2007 and 2008, 9.87 and 1.01% of samples contained less than 10^6 CFU/g of *Lb. acidophilus* at the end of consumption, while in other years they did not find any sample with number lower than 10^6 CFU/g (**Figure 2**).

The mechanisms of health-improving properties of probiotics are still not completely understood, but their anti-carcinogenic and anti-mutagenic activity, the suppression of allergies, reduction of serum cholesterol level and reduction in blood pressure are known [12, 80, 92, 93]. *Lb. bulgaricus* and *S. thermophilus* are able to ferment lactose, so they have beneficial effects for people suffering from lactose intolerance [94]. *Lb. rhamnosus* GG, *B. lactis* Bb-12, *Lb. acidophilus*, *Lb. casei* Shirota or *Lb. reuteri* have beneficial effects against acute diarrhoea caused by rotavirus, in treatment, shortening or preventing of this disease [95–97]. The administration of *S. boulardii* as non-pathogenic biotherapeutic yeast plays essential role in the treatment or prevention of antibiotic-associated diarrhoea caused by *C. difficile* [83, 98–101]. Probiotics, especially *Lb. acidophilus*, *Lb. plantarum*, *Lb. rhamnosus* and *Bifidobacterium*, are able to reduce faecal enzyme activity which converts procarcinogens into carcinogens (β -glucuronidase, azoreductase, urease, nitroreductase and glycocholic acid reductase) due to short-chain fatty acids production and may thus contribute to a decreasing risk of colorectal carcinoma [79, 102]. Other potential mechanisms for probiotics-induced anti-carcinogenic activity are described in the works of Commune et al. [92] and Faghfoori et al. [103], respectively.

4.2. Fermented cereals and pseudocereals functional products

Recently, there is an explosion of consumer's interest in functional foods; therefore, a key priority for food industry is the development of such products with a high quality and safety [104]. The aim of these products is to have beneficial effect on host health affecting gut microbial composition subsequently with reducing the risk of chronic diseases [105]. Cereals have been investigated in recent years regarding their potential use in the production of functional foods [106].

Possible application of cereals in functional food can be summarized as follows:

- as fermentable substrates for the growth of probiotic bacteria (lactobacilli, bifidobacteria);
- as prebiotics due to their content of non-digestible oligosaccharides (galacto-oligosaccharides and fructo-oligosaccharides);
- as dietary fibre promoting beneficial effects on human host;
- as encapsulation matter for probiotics to enhance their stability [104, 107].

Cereals have been and still are one of the most important sources of human diet [108] and are grown over 73% of total harvest area [109]. A number of cereals are grown in different

countries, including wheat, barley, oat, corn, rye, rice and millet, particularly important from an economical point of view. According to FAO's latest forecast, cereal production in 2015 stands at close to 2525 million tonnes but is still 1.4% below than the record in 2014 [110]. Cereal grains and their derivatives represent an important nutritive component both in developed and in developing countries [111]. They are considered as one of the most important sources of dietary proteins, carbohydrates (starch and fibre), vitamins (B group) and minerals for people all over the world [112].

4.2.1. Nutritional value of cereals

Cereal grains are primarily a source of carbohydrates, and thus a good source of energy [113]. They form about two-thirds up to three-quarters of dry matter [114]. Monosaccharides are the basic components of oligo- and polysaccharides and are most represented in the forms of hexoses (fructose, glucose and galactose) and pentoses, arabinose and xylose [115]. Starch, the major component of cereal grains, occurs in starch granules of different sizes in endosperm.

Within common varieties, 25–27% of starch is presented as amylose and 72–75% represents amylopectin. However, in cereals a portion of the presented starch is not digested and absorbed in the small intestine. This is referred to as resistant starch and it appears to act in a similar way to a dietary fibre [113]. A wide variety of biochemical processes occur in cereals during fermentation as a result of lactic acid bacteria. Fermentation process itself may lead to an increase in the content of reducing sugars, which was confirmed also in a study by Marko et al. [116]. Simple carbohydrates are metabolized directly to organic acids and the glucose as a final product of starch metabolism is utilized immediately [116]. Lambo et al. [117] described the decrease in starch content during fermentation of barley with lactobacilli.

Cereals are in general good sources of proteins. The proportions of essential amino acids and their digestibility mainly determine protein nutritional quality. Because of different production systems, environmental factors, as well as genotype, it is difficult to obtain comparative values of protein contents of different cereals. Thus, ranges of 5.8–7.7% of protein on a dry weight have been measured for rice, 8.0–15.0% for barley and 9.0–11.0% for maize. The amount of lysine, which is the limiting amino acid for all cereals, varies between species with the highest values in oat and rice and lowest in wheat and maize [118]. The most represented is glutamic acid in the form of glutamine [119]. Degradation and depolymerization of proteins during fermentation process depend not only on the metabolic activity of presented bacteria but also on enzymes that naturally occurred in cereals. Peptides are converted to amino acids by the activity of lactic acid bacteria by the specific intracellular peptidases that are subsequently converted to the specific products influencing the aroma and taste of final products [120]. Antony and co-workers [121] in their study pointed out that the fermentation process does not generally significantly change the total protein content of cereals. However, in the case of yeast corn fermentation, Cui et al. [122] found a significant increase ($P < 0.05$) in the total protein content.

Lipids are only a minor component of cereal grains with the amount varying from 1.7 to 7.0% on a dry mass basis, dependent on the type of cereal grain. The germ is the richest source of lipids. In particular, cereals are rich in essential fatty acids and contain only trace amounts of

saturated fatty acids [123]. Oxidation of lipids during fermentation process creates volatiles that contribute to the flavour of final products. Linoleic, oleic and linolenic acids are oxidized by lipoxygenases by forming hydroperoxides that are formed to aldehydes [124]. Aldehydes are converted to alcohols by alcohol dehydrogenases during fermentation process [125]. Antony et al. [121] in their study did not record any changes in the total lipid content during the millet fermentation with the endogenous microorganisms.

Cereals may contribute to vitamin intake due to the presence of most B-vitamins and appreciable amounts of vitamin E. Wholegrain cereals also contain considerable amount of calcium, magnesium, iron, zinc, as well as lower levels of many trace elements, for example, selenium. The content of minerals ranges from 1.0 to 2.5% [113, 126]. Cereals contain relatively high levels of phytate (0.2–1.4%), concentrated mostly in the aleurone layer, which can bind minerals and there is an evidence of its decreased absorption in the presence of phytate, so minerals are not available to microorganisms. However, at a pH values less than 5.5, phytates are hydrolysed by endogenous phytases, thus minerals are released from the complex [9]. In our investigation, changes in chemical composition of maize flours before and after expiry date were determined (**Table 3**). The percentage of starch and reducing sugars is one of the most important aspects showing the suitability of the tested substrate in fermentation technologies. A decline in the content of reducing sugars (60.1%) and starch (7.9%) was observed. Matejčková and co-workers [30] recorded a decline of reducing sugars in amaranth flours before and after expiry date of about 31% in their study.

In comparison to milk and dairy products, the nutritional quality of cereals and their products is sometimes inferior, or poor. The reason is the lower protein content in comparison to milk, limitations in the amounts of certain amino acids, notably lysine, and the presence of antinutritive compounds (phytic acid, tannins and polyphenols) and a coarse nature of grains [7, 127]. Cereals typically undergo a range of processes that change the nutritional content. Milling is the main process associated with cereals; also, extrusion is used to produce a variety of different types of products [128].

	Proteins	Lipids	Starch	Reducing sugars
Corn flour 1	3.21 ± 0.00	1.59 ± 0.03	68.71 ± 0.12	4.24 ± 0.01
Corn flour 2	4.46 ± 0.07	2.49 ± 0.00	63.30 ± 0.24	1.69 ± 0.01

Corn flour 1 (before expiry date), corn flour 2 (after expiry date), the results are means ± standard deviation of two determinations.

Table 3. Chemical composition of maize flours before and after expiry date (%).

Helland et al. [106] studied the growth and metabolism of four selected probiotic strains in rice- or maize-based puddings with milk or water. All four tested strains showed good growth and survival in cereal-based puddings.

4.2.2. Fermented cereal and pseudocereal food and beverages

Fermented food and beverages are defined as those products that have been subjected to the effect of microbial enzymes, particularly amylases, proteases and lipases that causes biochemical transformation of polysaccharides, proteins and lipids to non-toxic variety of desirable products with tastes, aromas and textures attractive to a consumer [4, 7]. Microorganisms responsible for the fermentation process may be presented naturally in the substrate, or may be added as a starter culture [9].

Traditional cereal- and pseudocereal-fermented products are made of various kinds of substrates all over the world, mainly widespread in Asia and Africa. Fermentation may have multiple effects on the nutritional value of food [129].

The development of non-dairy-fermented products is a challenge to the food industry by producing high-quality functional products. The main aims of cereal fermentation can be summarized as follows:

- preservation, which relies mainly on acidification (production of lactic, acetic and propionic acid) and/or alcoholic production often in combination with reduction of water activity [130];
- enhances the safety of final products by the inhibition of pathogens [131];
- affecting sensory properties (taste, aroma, colour and texture);
- improves the nutritional value by removing antinutritive compounds (phytic acid, enzyme inhibitors, tannins and polyphenols) and enhances the bioavailability of components;
- reduces the level of carbohydrates as well as non-digestible poly- and oligosaccharides [9].

Cereal fermentations affected by characteristic variables include the following:

- the type of cereal determining the content of fermentable substrates, growth factors, nutrients, minerals, nitrogen sources and buffering capacity;
- duration and temperature of fermentation process;
- water content;
- additional components (sugars, salt and exposure to oxygen);
- sources of amyolytic activity to gain fermentable sugars from starch or other polysaccharides [9, 132].

Fermented cereal-based products are prepared in different parts of the world, mainly in developing countries—Asia and Africa, in combination with legumes to improve overall protein quality of the final fermented products [7]. Petruřáková and Valík [133] evaluated the growth and metabolic activity of *Lb. rhamnosus* GG during fermentation of leguminous porridges. Cell density during 21-day cold storage was stable except whole soybean, yellow pea and red bean. Metabolic activity of observed strain caused decrease in pH values to the final 5.6–6.0 and subsequently during cold storage decreased. Fermented products are usually

prepared in the form of beverages, gruels or breakfast meals. Most of the fermented products are made in Asia (soy sauce), India (idli and dosa) and in the Middle East (kishk). In America, as a basic raw material for the production of cereal-fermented foods, corn is used, in products such as tescüino (alcoholic beverage of Mexico) or jamin-bang-bread made in Brazil [134]. An overview of traditional fermented food and beverages is summarized in **Table 4**.

Fermented food/country	Raw material/substrate	Microorganism
Idli—South India, Sri Lanka	Rice	<i>Leuconostoc mesenteroides</i> , <i>Enterococcus</i> , <i>Torulopsis</i>
Dosa—India	Rice	<i>Leuconostoc mesenteroides</i> , <i>Streptococcus faecalis</i> , <i>Torulopsis candida</i>
Kishk—Egypt, Syria	Wheat, milk	<i>Lactobacillus plantarum</i> , <i>Lb. casei</i> , <i>Lb. Brevis</i> , yeasts.
Tarhana—Turkey	Wheat, yoghurt	<i>Lactobacillus bulgaricus</i> , <i>Streptococcus thermophilus</i> , <i>Saccharomyces cerevisiae</i>
Ogi—West Africa	Maize, millet, sorghum	<i>Lactobacillus plantarum</i> , yeasts, moulds
Pozol—Mexico	Maize	Moulds, yeasts, bacteria
Injera—Ethiopia	Sorghum, maize	<i>Candida guilliermondii</i>
Sake—Japan	Rice	<i>Saccharomyces sake</i>
Bouza—Egypt	Wheat	LAB
Boza—Albania, Turkey	Wheat, millet	LAB, <i>Saccharomyces cerevisiae</i> , <i>Leuconostoc</i>
Mahewu—South Africa	Maize	<i>Lactococcus lactis</i>
Chicha—Peru	Maize	<i>Aspergillus</i> , <i>Penicillium</i> , yeasts, bacteria
Uji—Kenya, Uganda	Maize, millet, sorghum	<i>Lactobacillus plantarum</i> , <i>Leuconostoc mesenteroides</i>

Table 4. Overview of traditional fermented products and beverages [7, 135].

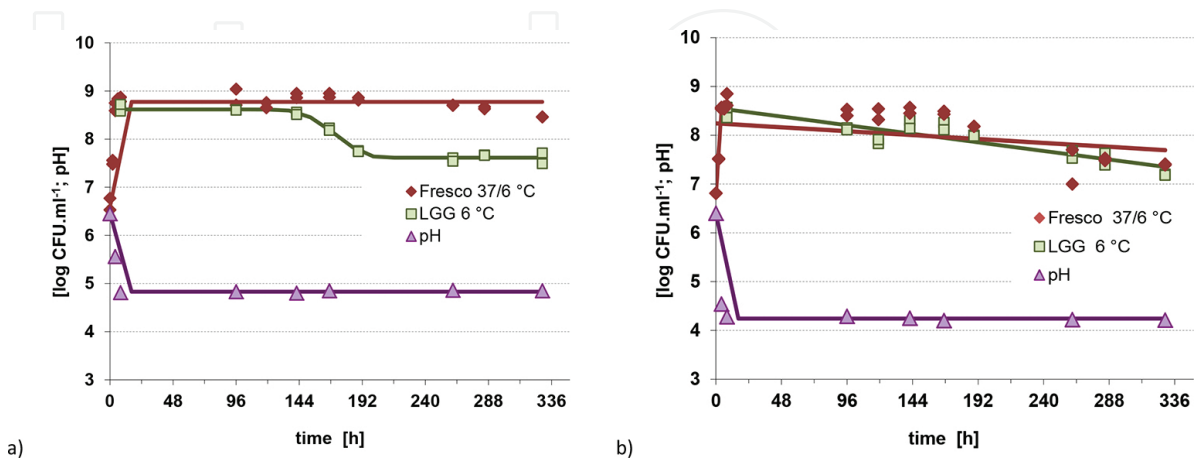


Figure 3. Presumptive counts of the cocci from Fresco DVS 1010 culture and *Lb. rhamnosus* GG (LGG) content in milk-based (a) and water-based (b) maize mashes.

As an example, the growth of Fresco DVS 1010 culture at 37 °C and the survival of probiotic strain *Lb. rhamnosus* GG (6 °C) in milk- and water-based maize mashes with sucrose are demonstrated in **Figure 3** as well as the growth parameters in **Table 5**. In general, the obtained maximal counts of monitored Fresco DVS 1010 culture after 8 h of fermentation process were $N = 10^8$ – 10^9 CFU ml⁻¹ from initial $N_0 = 10^6$ – 10^7 CFU ml⁻¹, which shows the suitability of tested sweet corn mashes for the growth and survival of lactic acid bacteria. During the refrigerated storage at 6°C (**Table 6**), a decline in the number of probiotic strain *Lb. rhamnosus* GG was observed, but not under the levels of 10⁶ CFU ml⁻¹ necessary from the legislation point of view.

Microorganism	Substrate corn flour	Gr_f (log CFU ml ⁻¹ h ⁻¹)	λ (h)	k_{pH} (h ⁻¹)
Fresco DVS 1010	Milk	0.522	–	-0.231
	Milk + caramel	0.446	–	-0.345
	Milk + chocolate	0.563	–	-0.172
	Water	0.445	–	-0.481
	Water + caramel	0.508	0.59	-0.298
	Water + chocolate	0.540	–	-0.462

Gr_f , growth rate; λ , lag-phase duration; k_{pH} , rate constant for the decrease of pH. The growth data were fitted using DMFit tool, kindly provided by Dr. J. Baranyi.

Table 5. Growth parameters of Fresco culture, 8-h fermentation at 37°C in maize mashes.

In botanical terms, amaranth, quinoa and buckwheat are not true cereals. They are dicotyledonous plants, and thus not cereals (monocotyledonous). Their seeds are in function and composition similar to true cereals, so they are referred as pseudocereals [136, 137]. Gluten-free pseudocereals increased attention worldwide, because they represent alternative to conventional gluten-containing cereals and industrially are used for the production of gluten-free products, especially for celiac patients. They enrich the nutrition of health people and contribute to their balanced diet. In comparison to cereals, pseudocereals are characterized by the increased availability of proteins, as well as its higher content. Moreover, pseudocereals are the major source of minerals and vitamins, and in comparison to cereals, the content of essential amino acid lysine is higher [138–141].

Due to its chemical composition, amaranth is considered as one of the most nutritious plants that is easy to grow and over 60 species of amaranth are known worldwide [142]. Grains are characterized with balanced composition of essential amino acids, especially lysine and methionine, higher content of proteins (15–17%) and starch (60–65%) [143, 144]. Compared to other cereals, the fat content is higher, ranging from 7 to 8%. Overall, amaranth is a good source of vitamins (riboflavin, niacin and vitamin E) and minerals such as calcium and magnesium [138]. A growing number of studies have investigated the usage of amaranth in cereal technology not only in the production of nutrient-rich gluten-free products but also to enrich diet of health people [145]. Several studies have also reported the possibility to enrich wheat-based products with amaranth to improve the quality and overall nutritional value of final

products [140]. Matejčková et al. [146] confirmed in their study the growth of probiotic and potentially probiotic strains (*Lb. acidophilus* 145, *Lb. rhamnosus* GG, *Lb. rhamnosus* VT1 and *Lb. paracasei* subsp. *paracasei*) in water- and milk-based amaranth mashes. The same authors [30] studied the growth and survival of probiotic strain *Lb. rhamnosus* GG in flavoured amaranth mashes, which were acceptable not only from the microbiological point of view but also from the sensory evaluation. Kocková and Valík [147] evaluated the suitability of selected cereals and pseudocereals for the development of new probiotic foods fermented by *Lb. rhamnosus* GG. The highest growth rate was calculated in the case of amaranth flour (0.589 log CFU g⁻¹ h⁻¹) and the longest lag phase was observed.

Substrate corn flour	k_d (log CFU ml ⁻¹ h ⁻¹)	λ (h)	N_0 (log CFU ml ⁻¹)	N_{end} (log CFU ml ⁻¹)
Milk	-0.0212	139.14	8.61	7.61
Milk + caramel	-0.0031	–	8.57	7.91
Milk + chocolate	-0.0200	–	7.91	7.04
Water	-0.0036	–	8.47	7.19
Water + caramel	-0.0033	141.78	8.27	7.65
Water + chocolate	-0.0093	–	7.47	7.03

k_d , rate constant for decrease of the *Lb. rhamnosus* counts; N_0 , initial counts; N_{end} , final counts after 14 days of storage period. The growth data were fitted using DMFit tool kindly provided by Dr. J. Baranyi.

Table 6. Parameters evaluating the behaviour of *Lb. rhamnosus* GG in fermented maize mashes during storage at 6°C when added after fermentation.

Substrate buckwheat flour	Gr_f (log CFU ml ⁻¹ h ⁻¹)	λ (h)	N_0 (log CFU ml ⁻¹)	N_{max} (log CFU ml ⁻¹)
Milk + vanilla	0.251	2.7	6.80	8.02
Milk + caramel	0.641	1.1	6.25	8.49
Milk + chocolate	0.332	3.0	6.74	8.32
Water + vanilla	0.275	2.4	6.93	8.48
Water + caramel	0.580	–	6.12	8.40
Water + chocolate	0.258	1.3	6.76	8.49

Gr_f , growth rate; λ , lag-phase duration; N_0 , initial counts; N_{max} , counts after storage period. The growth data were fitted using DMFit tool kindly provided by Dr. J. Baranyi.

Table 7. Growth parameters of *Lb. rhamnosus* GG in fermented buckwheat-flavoured mashes during fermentation at 37°C.

Together with amaranth, buckwheat and its products are studied in connection with celiac disease. Buckwheat was initially grown mainly in Asia and later has spread to Europe, Australia as well as to USA and Canada. The total carbohydrate content is 67–70%, of which

55% represents starch stored in the endosperm, as in common cereals. Buckwheat has a good content of thiamine, riboflavin and pyridoxine, and also represents a good source of minerals – magnesium, copper and potassium. It is characterized by a unique concentration of phytochemicals, in particular rutin, which has a positive effect on health especially in the prevention of cardiovascular diseases [148, 149]. Pelikánová et al. [109] evaluated the growth dynamics of *Lactobacillus* spp. in sweet buckwheat gruels. The population density of tested lactobacilli reached counts 10^8 – 10^9 CFU ml⁻¹ after 8 (10) h of fermentation, and after a 3-week-refrigerated storage period, the number of lactobacilli slightly increased except *Lb. acidophilus* 145. Liptáková et al. [87] in their study examined the pressed buckwheat products. The most suitable strain for fermentation was *Lb. rhamnosus* GG. Pressed fermented buckwheat water product with vanilla flavour was after 24 h of fermentation and after 5 days of storage evaluated with higher points according to the final evaluation of overall sensory acceptance.

Substrate buckwheat flour	k_d (log CFU ml ⁻¹ h ⁻¹)	λ (h)	N_{end} (log CFU ml ⁻¹)
Milk + vanilla	0.0006	–	8.54
Milk + caramel	-0.0002	–	8.42
Milk + chocolate	0.0009	–	8.89
Water + vanilla	0.0000	–	8.38
Water + caramel	-0.0002	–	8.41
Water + chocolate	0.0000	–	8.49

k_d , rate constant for decrease of the *Lb. rhamnosus* counts; λ , lag-phase duration; N_{end} , final counts after 21 days of storage period. The growth data were fitted using DMFit tool kindly provided by Dr. J. Baranyi.

Table 8. Parameters of *Lb. rhamnosus* GG in fermented buckwheat-flavoured mashes during storage at 6°C.

As for the example, growth and fermentative metabolism of probiotic strain *Lb. rhamnosus* GG in buckwheat mashes with caramel/vanilla/chocolate flavour is summarized in **Tables 7** and **8**. Investigated probiotic strain showed sufficient growth and survival in prepared flavoured mashes with the growth rates ranging from 0.251 to 0.641 log CFU ml⁻¹ h⁻¹. At the end of cold storage, densities of *Lb. rhamnosus* GG maintained above the minimum limit of 10^6 CFU ml⁻¹.

The interest of consumers in fermented cereal- or pseudocereal-based products is growing. The development of non-dairy-fermented products including probiotics may lead to enrichment of the diet in patients suffering from celiac disease, people with allergies, or intolerances, but it may contribute to the balanced diet of healthy subjects [149]. If the cereal or pseudocereal products are presented with an attractive sensory taste, it may represent a suitable option for the development of new probiotic foods. Thus, in our study we evaluate the overall sensory acceptability of maize-flavoured (chocolate/caramel) mashes (**Figures 4** and **5**). The overall acceptability was evaluated from 2.80 to 3.30 (four-point scale) that indicated pleasant acceptance except caramel water mash (2.56). Kocková and Valík [135] noted negative effect of a 21-day storage period on overall acceptability buckwheat product with salt fermented by probiotic strain *Lb. rhamnosus* GG from values 3.31 to 2.44. In our study, no decline of overall acceptance during storage period was observed.

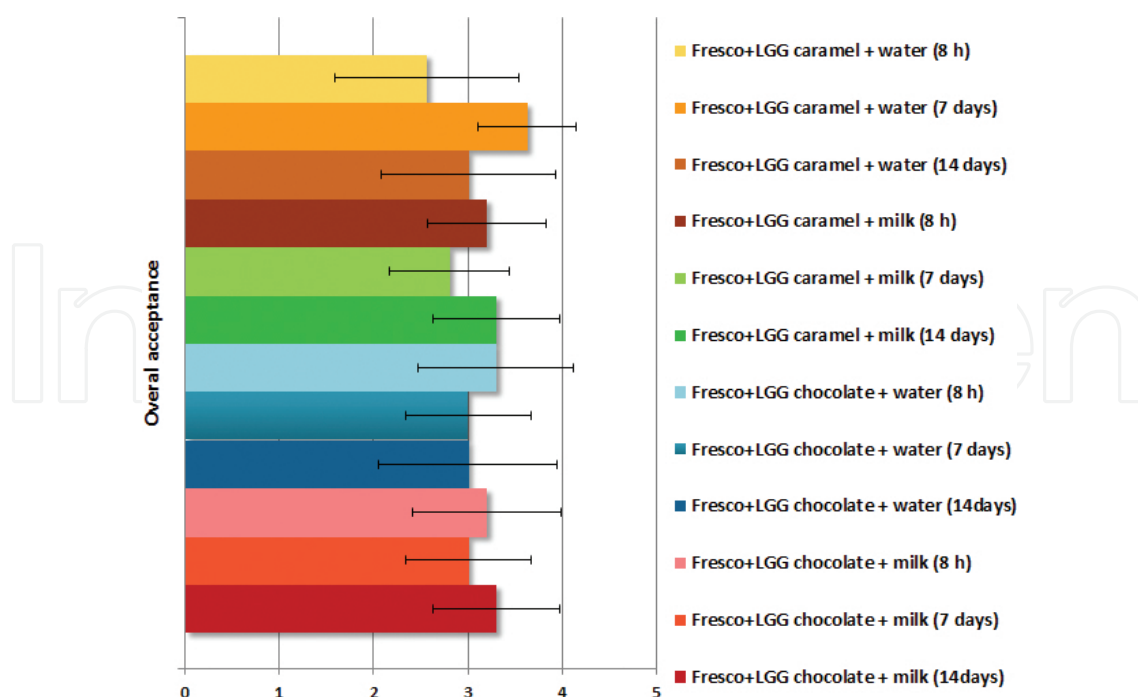


Figure 4. Evaluation of overall acceptability maize caramel/chocolate mashes (LGG—*Lactobacillus rhamnosus* GG).



Figure 5. Photo-documentation of flavoured final maize products.

5. Conclusion

Sustainable diets and cultured consumer interests, for example, in personal health, represent the main driving forces for the development of new functional foods in the world. Throughout the world, many fermented foods that are produced cover a wide range of substances and microorganisms. Ensuring high quality and safety for such a product requires deep under-

standing of fermentation process, types and roles of microorganisms used and specific final product characteristics. Lactic acid bacteria are the alternatives of food biopreservation primarily due to the production of weak organic acids and other inhibitory substances in combination with lowering pH and reduction of redox potential. LAB and their metabolites are able to slow or inhibit the growth of undesirable bacteria, yeasts and toxigenic fungi in food. There is evidence that LAB are also able to reduce the gluten content of cereals that represents increasing problem for 0.5–1% of population worldwide. Many lactic acid bacteria and other microbial strains such as *E. coli* Nissle, *B. cereus*, *B. subtilis* or *S. boulardii* belong to the probiotics with documented positive effects on human health.

The development of fermented cereal- or pseudocereal-based products supplemented with probiotics represents an available alternative to milk products and may lead to enrichment of the diet of people suffering from celiac disease, allergy to milk proteins, lactose intolerance people or otherwise metabolically handicapped consumers, but it may also contribute to a balanced diet of healthy subjects.

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