Acta Agraria Kaposváriensis (2015) Vol 19 No 1, 65-74.
Kaposvári Egyetem, Agrár- és Környezettudományi Kar, Kaposvár

How to maintain the effective levels of probiotics throughout the shelf life in yoghurt: A review

É. Varga-Visi, G. Pápai
Kaposvár University, Faculty of Agricultural and Environmental Sciences, H-7400 Kaposvár, Guba S. u. 40.

ABSTRACT

Retention of probiotic functionality throughout the entire shelf life of probiotic yoghurt can be a challenge for manufacturers. The review was aimed to interpret the factors that have an influence on the growth of probiotic microorganisms in fermented dairy foods, especially in yoghurts. Compatibility between strains is important both for the manufacture and storage of the product. Inoculation with commercial and probiotic cultures results in complex interaction among strains that is advisable to consider during strain selection and setting of fermentation conditions. Processing steps like cold ripening and storage of yoghurt can represent a threat for the viability of probiotic bacteria as an adverse environment is present. The most important factors that can be a matter of concern are the presence of oxygen, low pH and cold stress. Strategies to eliminate the drawback of these conditions based on chemical and enzymatic methods and technological developments with respect to packaging. Growth is strongly influenced by ingredients involved into the food matrix. Incorporation of prebiotics can improve the viability of probiotics during manufacturing and storage of yoghurt and can contribute to the achievement and maintenance of effective cell numbers to confer beneficial effects for the host. The prerequisite of the effective use of prebiotics is their chemical stability under the applied manufacturing conditions.
(Keywords: probiotic viability, bifidobacteria, prebiotics, inulin, yoghurt storage)

INTRODUCTION

The most widely accepted definition of probiotics is that "probiotics are live microorganisms, administrated in certain quantities that confer health benefits to the host" (*FAO/WHO,* 2001). Their positive effect on gut microbiota and gut-associated lymphoid system (GALT) can be utilized if they ingested in adequate amounts (*Granato et al.,* 2010; *Divya et al.,* 2012; *Saad et al.,* 2013). Probiotics can be incorporated in both foods and dietary supplements. While activity of strains is stopped due to low water activity values in tablets or capsules which contain freeze dried cell powders, their microbiological life cycle continues in food matrixes and the number of viable cells is changing during production and storage of foods. The retention of viability of the strains is maybe the greatest challenge in the production of probiotic foods (*Divya et al.,* 2012).
Fermented milk products are excellent carrier foods for probiotic microorganisms, moreover yoghurt is considered to be the most popular among them (*Divya et al.,* 2012; *Pandey & Mishra,* 2015). International Dairy Federation (IDF) defined that a product could be declared as probiotic if the number of viable probiotic cells is more than 10^7 CFU/g in the time of consumption, that is, up to the date of minimum durability (*Divya*

et al., 2012). In order to achieve the adequate cell number for health effects the applied strains should be compatible with each other. To accomplish this, one should be aware of the interaction between the members of conventionally used yoghurt starter and probiotic starters and accomplish fermentation in such a way that utilize the advantages of possible synergisms and avoid the disadvantageous effects of antagonisms on probiotic cell counts. In addition the sensitivity of strains can also differ regarding ranges of environmental conditions like temperature, redox potential or pH. Storage conditions of yoghurt could especially exert negative effects on probiotics in this respect (*Granato et al.,* 2010). Quality control results of commercialised products showed an adequate enumeration at the time of purchase but counts dropped under the required level before the expiry date as probiotic lactobacilli and bifidobacteria showed a decline in their viability during storage (*Paseephol & Sherkar,* 2009; *Jayamanne & Adams,* 2006).

Prebiotics can enhance the viability of probiotics both in the gastrointestinal tract (*Charalampopoulos & Rastall,* 2012; *Divya et al.,* 2012; *Al-Sheraji et al.,* 2013; *Saad et al.,* 2013) and in foods (*Lourens-Hattingh & Viljoen,* 2001). Individual oligosaccharides have different capabilities to improve of viability of probiotic strains during the shelf life of yoghurt. Prebiotics are being present during the operation units of yoghurt manufacture therefore their chemical stability has to be evaluated under the applied conditions. In the case of partial or total decomposition during processing they can loss their ability to selectively support the viability of probiotic bacteria.

The review is aimed to summarize the factors that can promote or hamper the development and retention of effective viable probiotic cell counts during the processing and storage of probiotic and symbiotic yoghurts.

Interactions between strains during manufacture of probiotic yoghurt

Yoghurt is resulted from the fermentation of milk with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. These species cannot be considered as probiotics (*Espírito Santo et al.,* 2011). The most often used probiotic genera in yoghurt are *Lactobacillus* and *Bifidobacterium* (*Holzaphel et al.,* 1998; *Charalampopoulos & Rastall,* 2012; *Saad et al.,* 2013; *Al-Sheraji et al.,* 2013). Compatibility among strains is important in the manufacture and storage of the product, and also following consumption as it may exert an effect on the degree of adherence to the intestinal mucosa (*Collado et al.,* 2007).

High populations of *L. delbrueckii* ssp. *bulgaricus* can reduce drastically the enumeration of probiotic *L. acidophilus* with the production of hydrogen peroxide that can cause a so called "acidophilus death" (*Hull et al.,* 1984). This antagonism seems to be mutual because the bacteriocin of *L. acidophilus* Acidophilicin LA-1 proved to be active against more strains of *L. delbrueckii* ssp. *bulgaricus* (*Dave & Shah,* 1997). Moreover, uncontrollable growth of strains of *L. delbrueckii* ssp. *bulgaricus* can cause an over-acidification of yoghurt (*Kneifel et al.,* 1993) that can be intolerable to bifidobacteria being highly sensitive to acidic conditions (*Lourens-Hattingh & Viljoen,* 2001; *Sanz,* 2007).

In summary, *L. delbrueckii* ssp. *bulgaricus* could exert antagonistic effects towards both bifidobacteria and *L. acidophilus* that can be involved in the production of probiotic yoghurts. The other participant of the conventionally used yoghurt culture may have an opposite role. Bifidobacteria are strictly anaerobic, whereas *Str. thermophilus* acts as an oxygen scavenger therefore it improves the viability of *Bifidobacterium* spp. (*Ishibashi & Shimanura,* 1993). This observation was supported by the fact that *B. lactis* inoculated in milk as binary culture (co-culture) with *Str. thermophilus* had higher counts than the

pure culture of *B. lactis* in the same substrate both after processing and one-week storage (*Oliveira et al.,* 2011).

Strains of probiotic cultures could support each other in growth owing to synergistic effects. Some part of bifidobacteria lacks of proteolytic activity e.g. *Bb. bifidum.* They could be provided with the necessary growth factors in co-fermentations with lactobacilli with proteolytic activity like *L. acidophilus* (*Hansen, 1985; Klaver et al.,* 1993) or *L. delbrueckii* ssp. *bulgaricus* and *Str. thermophilus* (*Dave & Shah,* 1998).

Strains of traditional starters and that of probiotic cultures could have synergistic effect per se but it might be disadvantageous if these bacterial cell cultures are fermented together owing to the antagonistic effects of *L. delbrueckii* ssp. *bulgaricus.* Addition of prebiotic cultures following acidification with the commercial yoghurt culture is not an adequate solution because fermentation is not restricted to the time until the pH reaches the isoelectric point of casein as the post-ripening continues during cold storage. *Gilliland and Speck* (1977) added probiotic cultures following common yoghurt fermentation and they observed a rapid cell count decline of *L. acidophilus* during cold storage. Nevertheless, when traditional culture and *L. acidophilus* were cultured together from the initial point of fermentation, *L. acidophilus* presumably developed an ability to split hydrogen peroxide produced by *L. delbrueckii* ssp. *bulgaricus* (*Hull et al.,* 1984; *Lourens-Hattingh & Viljoen,* 2001) that may contribute to its better survival during cold storage. In the case of bifidobacteria the presence of oxygen eliminating *Str. thermophilus* may be beneficial. Counts of *B. lactis* were higher in a mixed culture in which both *Str. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were included related to enumeration of its pure culture (*Oliveira et al.,* 2011) therefore simultaneous inoculation of *Bifidobacterium* spp. could be suggested during production of probiotic yoghurt.

Effect of environmental conditions on viability of probiotics during manufacture and storage of yoghurt

In the case of mixed fermentations differences in the optimal inoculation temperatures of strains have to be considered. Conventionally used yoghurt starters have an optimum temperature for lactic acid production of approximately 43 °C whereas the optimum growth temperature of bifidobacteria is 37 °C. In order to improve the growth rate of probiotic strains fermentation temperatures between 37 °C and 40 °C were suggested to be effective (*Kneifel et al.,* 1993).

However, several conditions are hard to optimize for the better survival of probiotics. The most important factors that can be a matter of concern are presence of oxygen, low pH and cold stress (*Sanz,* 2007; *Granato et al.,* 2010). Processing steps of yoghurt can represent a threat for the viability of probiotic bacteria as an adverse environment is present at the end of fermentation during cold ripening and storage. Manufacturers apply several strategies to eliminate these disadvantageous conditions e.g. use packaging containers of low oxygen permeability, select more acid-tolerant strains or trigger their adaptation, or microencapsulate probiotics (*Sanz,* 2007).

Probiotic bacteria prefer an anaerobic environment. The surface of their matrix is connected to air when yoghurt is processed i.e. stirring is a unique operation when oxygen can be incorporated into the yoghurt. The positive effect of *Str. thermophilus* on the viability of bifidobacteria via elimination of oxygen has been described in the previous section. An enzymatic method was developed to eliminate the remaining oxygen after packaging with glucose oxidase (*Cruz,* 2010). A chemical alternative was to keep the matrix in reduced state with ascorbate (*Dave & Shah,* 1998; *Zhao & Li* 2008). Bifidobacterium strains can be protected from oxygen via microencapsulation

(*Talwalkar & Kailasapathy,* 2003). Yoghurts are usually stored for more weeks before consumption therefore the oxygen permeability of packaging material can be important with respect to the viability of anaerobic bifidobacteria. Development and application of appropriate packaging materials and systems are necessary to maintain the required levels of probiotics throughout the shelf life in order to guarantee the therapeutic potential of product **(***Talwalkar & Kailasapathy,* 2004; *Cruz et al.,* 2007).

Strategies to eliminate the drawback of low pH on probiotic count can be the selection of acid tolerant strains, promoting stress adaptation, prevention of over-acidification with chemical neutralization of media or depress the fermentation of strongly acidifying strains. The acid tolerance of *Bifidobacterium* spp. is low in general but the toleration limit is strain-dependent. Strains derived from animal sources usually survive better the acidic conditions than those derived from human gastrointestinal tract. The reported pH values which caused growth inhibition were different among strains with the agreement that pH values lover than 4.6 led to the decline in case of most bifidobacteria (*Martin & Chou*, 1992; *Lankaputhra & Shah,* 1995; *Reilly & Gilliland,* 1999; *Lourens-Hattingh &Viljoen,* 2001; *Sanz,* 2007). Among bifidobacteria *B. animalis* was reported to have the best ability to survive under acidic conditions (*Sanz,* 2007).

Most lactobacilli are neutrophilic and their growing optimum is between pH 5 and 9 with the exception of few *Lactobacillus* and *Leuconostoc* species (*Granato et al.,* 2010). Nevertheless, lactic acid bacteria and bifidobactera have some capabilities to express their acid tolerance that can be induced via facing the acid stress for a short time period. The stress adaptation is achieved with the short exposure to sub-lethal factors resulting tolerance to subsequent lethal conditions **(***Sanz,* 2007, *Granato et al.,* 2010).

Acidification can be hampered with the addition of alkaline hydrolysing salts to the media, e. g. sodium citrate or calcium carbonate to neutralize lactic acid (*Zhao & Li,* 2008). The growth of *L. delbrueckii* ssp. *bulgaricus* can be suppressed and the over-acidification can be avoided if the storage temperature is less than 3–4 °C (*Kneifel et al.,* 1993). Nevertheless, the ratio of bifidobacteria and *L. acidophilus* can change as *Bifidobacterium* spp. are less tolerant to lower temperatures owing to cold stress resulting change in membrane fluidity, DNA/RNA functions and enzymatic activity (*Hughes & Hoover,* 1995; *Corcoran et al.,* 2007).

The growth of probiotic bacteria with limited proteolytic activity like some bifidobacteria can be supported with available sources of nitrogen. Dairy matrix can be supplemented with whey derivatives, hydrolyzed proteins or free amino acids and viability of probiotic strains can be enhanced. Parallel these authors also described an improvement in structural properties like firmness and syneresis (*Antunes et al.,* 2005; *Zhao & Zhang,* 2006). However, the economics of this step should be considered and the quantity of addition should be optimized (*Granato et al.,* 2010).

Application of prebiotics in yoghurt with special respect to the viability of probiotics throughout the self-life of product

The terms of "dietary fibre" and "prebiotic" are similar in that respect that both of them describes carbohydrates that resist to mammalian enzymes and gastric juice but can be partially fermented by gut bacteria. Perhaps the main difference between these groups is that prebiotics have been proved to selectively support the fermentation in the large intestine towards the beneficial microorganisms of the host. The combination of probiotics and prebiotics in foods results synbiotics. In symbiotic products the delivery and implantation of living organisms into the microbiota of gastrointestinal tract is improved with their selective substrates (*Divya et al.,* 2012; *Al-Sheraji et al.,* 2013).

The most often used types of prebiotics are galactooligosaccharides (GOS), fructooligosaccharides (FOS), inulin and its hydrolizates (*Al-Sheraji et al.,* 2013), whereas isomalto-oligosaccharides (IMO), xilo-oligosaccharides (XOS), soybean oligosaccharides (SOS) and resistant starch are emerging prebiotics (*Divya et al.,* 2012; *Charalampopoulos & Rastall,* 2012; *Saad et al.,* 2013). The group of prebiotics is continuously increasing. Nowadays prebiotics are included in food products primarily to promote a balanced gut microbiota. Initially their application started as these carbohydrates can improve the techno-functional properties of foods like viscosity, emulsification capacity, gel formation and colour. Prebiotics can be used instead of those food technological additives that do not have an advantageous effect on health (*Zimeri & Kokini,* 2003; *Al-Sheraji et al.,* 2013; *Saad et al.,* 2013). Inulin and FOS can be used to restore the textural and organoleptic properties of low fat yoghurts (*Ramchandran & Shah,* 2010). These prebiotics were reported to reduce syneresis and improve organoleptic properties with the development of mouthfeel especially in low-fat dairy products (*Franck,* 2002; *Aryana et al.,* 2007; *Kip et al.,* 2006). Prebiotics can contribute to the dietary fibre intake of human but in a negligible extent compared with those derived from the consumption of other sources like fruits and vegetables.

Prebiotics improve selectively the viability of advantageous indigenous bacteria, moreover, can also exert a synergic effect on probiotics in food products during manufacture and storage (*Lourens-Hattingh & Viljoen,* 2001). A substantial issue is whether the probiotic cell count is high enough in the time of ingestion to provide beneficial effects for the consumer. In this respect prebiotics can promote the development and maintenance of an adequate viable cell number of probiotic bacteria during the whole shelf-life of the product.

Inulin term covers a variety length of oligosaccharides containing β-2,1-linked fructosil moieties with terminal glucosyl residue. Inulins can be obtained by direct extraction from natural sources e.g. chicory or produced by chemical or enzymatic hydrolysis of polysaccharides or synthesis from disaccharides (*Charalampopoulos & Rastall,* 2012; *Saad et al.,* 2013). Varying composition may cause differences in their properties to facilitate the fermentation of probiotics therefore their detailed description is necessary in citing relevant results. The degree of polimerization (DP) of fructose molecules generally ranged from 2 to 60. High performance (HP) inulin products do not contain small molecular weight oligomers, their DP ranges from 11 to 60 with an average of 25. This abbreviation refers to their high potential of acting as a fat substitute to enhance fat-like creamy mouth-feel (*Roberfroid,* 1999) and does not refers to the degree of selective supplementation of probiotic strains.

In general, incorporation of inulin enhanced the enumerations of bifidobacteria to a greater extent than that of probiotic *Lactobacillus* spp. during processing and storage of fermented milk products (*Oliveira et al.,* 2011; *Ramchandran & Shah,* 2010; *Özer et al.,* 2005; *Roberfroid et al.,* 1998). Addition of 4% (wt/wt) "Beneo TM" inulin (DP=10) increased the cell number of *B. lactis* with almost one order of magnitude related to probiotic joghurt without inulin addition at the end of the manufacture, moreover this high level of CFU was maintained until the end of one-week storage. In the presence of inulin the cell count was above 10^8 CFU/ml throughout the first week, while enumeration was below this value without prebiotics. In the case of probiotic lactobacilli (*L. acidophilus* and *L. rhamnosus*) the prebiotic effect of inulin was not so emphasized, although tests showed significant differences in some cases the effect was approximately one-tenth less than for *B. lactis* (*Oliveira et al.,* 2011). Similar results were obtained when *L. acidophilus, L. rhamnosus* and *B. lactis* were fermented separately in dual

cultures (co-cultures) with *Str. thermophilus*, that is, in the presence of inulin the highest CFU increment was detected for *B. lactis* (*Oliveira et al.,* 2009a). However, bifidogenic effect of inulin was more emphasized in single strain cultures (*Oliveira et al.,* 2011) or in dual cultures (*Oliveira et al.,* 2009a) than in mixed cultures with similar strains used during yoghurt fermentation (*Oliveira et al.,* 2009b).

Raftiline HP® is an inulin obtained from hot water extract from chicory roots with DP more than 23. Its bifidogenic activity was confirmed as inclusion of 1% (wt/vol) of it in reconstructed skim milk (RSM) almost doubled the increment of CFU for *B. longum*. The effect of this inulin on *L. casei* and *L. acidophilus* was not significant in doses from 1 to 3% (*Ramchandran & Shah,* 2010), that is the effect on lactobacilli was not so emphasized, related to bifidobacteria, similar to "Beneo TM" (*Oliveira et al.,* 2011). Moreover, higher doses (2 and 3%) of Raftiline HP® did not result in further growth improvement in the case of *B. longum* (*Ramchandran & Shah,* 2010).

Paseephol & Sherkat (2009) detected a reverse effect i.e. various inulins had more capability to enhance the enumeration of lactobacilli related to bifidobacteria. Three types of inulins (medium chain DP=10 Raftilose® P95 and short chain DP=4 Raftilone® GR derived from chicory, moreover Jerusalem artichoke inulin DP=9) increased more effectively the cell count of *L. casei* than that of *Bb. bifidum*. However, the base medium of growth was carbohydrate-free MRS broth that was completely different related to previous experiments when the cultures were inoculated to milk or reconstituted milk (*Özer et al.,* 2005; *Oliveira et al.,* 2009a; *Oliveira et al.,* 2009b; *Oliveira et al.,* 2011; *Ramchandran & Shah,* 2010).

The growth of monoculture of *L. acidophilus* and *L. casei* on direct carbon deficient MRS medium was supported effectively by SOS, FOS and inulin while arabinogalactan based commercial products and β-glucans were less effective. Similar trends were observed for *B. animalis* on a carbon source deficient RCM base medium (*Su et al.,* 2007).

Growth of some *Lactobacillus* and *Bifidobacterium* strains were investigated on MRS broth in the presence of commercial FOS and inulin products, moreover purified GOS. The prebiotic activity of oligosaccharides was expressed as they contribute to the growth related to the same ratio (1% wt/vol) of glucose in the broth. Different types of prebiotics used as carbon source exerted very similar growth effects for bifidobacteria under investigation i.e. strains of *B. breve, B. infantilis, B. adolescentis, B. longum*. However, there were notable differences in growth of *Lactobacillus* ssp. when utilizing the same carbon source for each type of prebiotics applied. Moreover, results clearly indicated that the utilization of prebiotics can be strain-dependent as there were significant differences between *L. acidophilus* NCFM and *L. acidophilus* 33200 in the case of the growth effects of all FOS and inulin products, all of them supporting better the growth of NCFM strain (*Huebner et al.,* 2007).

Lactulose has been shown to be more effective prebiotics than inulin with respect to the growth of *Bb. bifidum* BB-02 and *L. acidophilus* LA-5 in yoghurt (*Özer et al.,* 2005). Amylose maize starch containing resistant starch (Hi-maize®) enhanced the enumerations of *L. acidophilus* and *L. casei* in freshly prepared yoghurt, related to prebiotic-free product. However, this type of resistant starch proved to be a less potent prebiotic than inulin as cell numbers following production were significantly higher when inulin was applied, related to Hi-maize®. Moreover, yoghurt samples were also investigated during cold storage for four weeks. The CFU values of products supplied with inulin practically did not change while the cell numbers in products produced with addition of amylose maize starch declined continouosly during storage and dropped to

the level of yoghurts without prebiotics at the second and the fourth level of storage for *L. casei* and *L. acidophilus*, respectively (*Donkor et al.,* 2007).

Chemical stability of prebiotics during processing of yoghurt

Prebiotics must be chemically stabile during food manufacture. Selective stimulation of beneficial microorganisms cannot be provided if these oligosaccharides are chemically altered, e.g. hydrolized to their sugar units. In the case of yoghurt thermal treatment of milk mixed with prebiotics is a requisite to meet the requirements of microbiological safety therefore possible deterioration of these substrates can be a matter of concern.

Stability of oligosaccharides was evaluated mostly in low-pH-buffered model systems and non-dairy food matrixes. In the case of fruit juices pasteurizing prior to packaging may generate losses. GOS proved to be stable at different sort of pasteurization processes in various fruit juices with acidic pH, while inulin and FOS partially hydrolyzed (*Charalampopoulos & Rastall,* 2012). In the case of yoghurt production there is no coexistence of low pH and high temperature as heat treatment is carried out before fermentation when the pH of the raw milk is near to neutral.

Huebner and co-workers (2008) applied not only acidic but also neutral condition in model solutions of inulin and FOS based commercial prebiotics. Buffered solutions were heat treated at 85 °C for 30 min. Inulin products Inulin-S and Raftiline HP derived from chicory proved to be stabile between pH values from 5 to 7 while in the case of FOS based Raftilose P95 authors did detect decline in the prebiotic activity related to control irrespectively to the pH of the solution. In this latter case partial hydrolysis of glycoside bonds was very likely based on the results of HPLC analysis. The decrease of prebiotic activity was proportional to the decrease of pH between pH 7 and 4, presumably hydrolysis is promoted by acid catalysis. However, if hydrolysis is not complete just partial, strains that can utilize oligosaccharides with lower degree of polimerization (DP) better, gain an advantage from this chemical alteration. Nevertheless, in the absence of knowledge of these deteriorations authors could drown wrong conclusions regarding oligosaccharid utilization of strains with respect to DP.

CONCLUSIONS

The viability of probiotics in yoghurt depends on several microbiological and environmental factors that are determined by processing technologies. Interaction between strains can be quite different in a real product in which at least two mixed cultures moreover real food matrix is present related to model experiments in which some of the typical strains are missing and/or fermentation is carried out in microbiological substrates. However, interaction network between individual participants of the microbiota in yoghurt can be explored with simultaneous experiments with single strains and co-cultures.

Manufacturers are intent on optimizing the producing parameters in order to obtain and maintain the required probiotic cell numbers up to the date of minimum durability, however, in yoghurt an adverse environment is present for probiotics throughout cold storing owing to factors like low temperature and pH and the viability of the cells can be threaten. Addition of prebiotic oligosaccharides to the food matrix can improve significantly the viability of strains. Nowadays several novel prebiotics are under investigation. Providing probiotic strains with potential candidates of prebiotics as single carbon sources in microbiological substrates can be a useful tool as the first step to

evaluate their utilization. In the case of positive response the next step could be the accomplishment of co-fermentation with commercial starters in milk.

There are several well designed studies for the investigation of survival of bifidobacteria and lactobacilli in milk supplemented with inulin. The effect of inulin on viability has been evaluated in mono- binary- and mixed cultures. There is sparse information on the effectiveness of other probiotics in the case of yoghurt. The utilization of emerging probiotics was evaluated mostly in microbiological substrates and not in foods.

The effect of pasteurization on the chemical stability of prebiotics was studied in model solutions and in acidic fruit based products. Based on the scarce existing data the food matrix has had an effect on prebiotic stability. Authors cannot obtain available information on the effect of heat treatments applied before fermentation of dairy foods on the integrity of prebiotics.

ACKNOWLEDGEMENTS

Gréta Pápai's PhD scholarship is granted by the Hungarian government.

REFERENCES

Al-Sheraji, S.H., Ismail, A., Manap, M.Y., S. Mustafa, S., Yusof R.M., Hassan, F.A. (2013): Prebiotics as functional foods: A review. *Journal of Functional Foods*, 5. 1542-1553.

Antunes, A.E.C., Cazetto, T.F., Bolini, H.M.A. (2005): Viability of probiotic micro-organisms during storage, postacidification and sensory analysis of fat-free yogurts with added whey protein concentrate. *Int. J. Dairy Technol.*, 58. 169-173.

Aryana, K.J., Plauche, S., Rao, R.M., McGrew, P., Shah, N.P. (2007): Fat-free plain yoghurt manufactured with inulins of various chain lengths and Lactobacillus acidophilus. *J. Food Sci*., 72. 79-84.

Charalampopoulos D., Rastall R.A. (2012): Prebiotics in foods. *Curr. Opin Biotech*., 23. 187-191.

Collado, M.C., Meriluoto, J., Salminen S. (2007): Development of new probiotics by strain combinations: is it possible to improve the adhesion to intestinal mucus? *J. Dairy Sci*., 90. 2170-2176.

Corcoran, B.M., Stanton, C., Fitzgerald, G., Ross R.P. (2007): Life under stress: the probiotic stress response and how it may be manipulated. Curr Pharm Design 14. 1382-1399.

Cruz, A.G, Faria, A.F.J.,Van Dender, A.G.F. (2007): Packaging system and probiotic dairy foods. *Food Res. Int*., 40. 951-956.

Cruz, A.G., Cadena, R.S., Walter, E.H.M., Mortazavian, A.M., Granato, D., Faria, J.A.F., Bolini H.M.A. (2010): Sensory analysis: Relevance for prebiotic, probiotic, and synbiotic product development. *Comp. Rev. Food Sci. F*., 9. 358-373.

Dave, R.I., Shah, N.P. (1997): Characteristics of bacteriocin produced by Lactobacillus acidophilus LA-1. *Int. Dairy J*., 7. 707-715.

Dave, R.I., Shah, N.P. (1998): Ingredients supplementation effects on viability of probiotic bacteria in yogurt. *J. Dairy Sci*., 81. 2804-2816.

Divya, J.B., Varsha, K.K., Nampoothiri, K.M., Ismail B., Pandey A. (2012): Probiotic fermented foods for health benefits. *Eng. Life Sci*., 12. 377-390.

Donkor, O.N., Nilmini, S.L.I., Stolic, P., Vasiljevic, T., Shah, N.P. (2007): Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. *Int. Dairy J*., 17. 657-665.

Espirito-Santo, A.P., Perego, P., Converti A. &Oliveira M.N. (2011): Influence of food matrices on probiotic viability. A review focusing on the fruity bases. *Trends Food Sci. Tech*., 22. 377-385.

Franck, A. (2002): Technological functionality of inulin and oligofructose. *Brit. J. Nutr*., 87, S287-S291.

Gilliland, S.E. & Speck, M.L. (1977): Instability of Lactobacillus acidophilus in yogurt. *J. Dairy Sci*., 60. 1394-1398.

Granato, D., Branco, G. F., Cruz, A.G., Faria, J.A.F. & Shah, N.P. (2010): Probiotic dairy products as functional foods. *Comp Rev Food Sci. F*., 9. 455-470.

Hansen, R. (1985): Bifidobacteria have come to stay. *North European Dairy Journal*, 3. 1-6.

Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U. & Huis in't Veld, J.H.J. (1998): Overview of gut flora and probiotics. *Int. J. Food Microbiol*., 41. 85-101.

Huebner J., Wehling R.L., Parkhurst A., Hutkins R.W. (2008): Effect of processing conditions on the prebiotic activity of commercial prebiotics. *Int. Dairy J*., 18. 287-293.

Huebner, J, Wehling, R.L., Hutkins R.W. (2007): Functional activity of commercial prebiotics. *Int. Dairy J*., 17. 770-775.

Hughes, D.B., Hoover, D.G. (1995): Viability and enzymatic activity of bifidobacteria in milk. *J. Dairy Sci*., 78. 268-276.

Hull, R.R., Roberts, A.V., Mayes, J.J. (1984): Survival of Lactobacillus acidophilus in yoghurt. *Aust. J Dairy Technol*., 39. 164-166.

Ishibashi, N., Shimamura, S. (1993): Bifidobacteria: Research and development in Japan. *Food Technol*., 47. 129-134.

Jayamanne, V.S., Adams, M.R. (2006): Determination of survival, identity and stress resistance of probiotic bifidobacteria in bioyoghurts. *Lett. Appl. Microbiol*., 42. 189-194.

Kip, P., Meyer, D., Jellema, R.H. (2006): Inulins improve sensoric and textural properties of low-fat yoghurts. *Int. Dairy J*., 16. 1098-1103.

Klaver, F.A.M., Kingma, F., Weerkamp, A.H. (1993): Growth and survival of bifidobacteria in milk. *Neth. Milk Dairy J*., 47. 151-164.

Kneifel, W., Jaros, D., Erhard, F. (1993): Microflora and acidification properties of yogurt and yogurt-related products fermented with commercially available starter cultures. *Int. J. Food Microbiol*., 18. 179-189.

Lankaputhra, W.E.V., Shah, N.P. (1995): Survival of Lactobacillus acidophilus and Bifidobacterium species in the presence of acid and bile salts. *Cultured Dairy Products Journal*, 30. 113-118.

Lourens-Hattingh, A., Viljoen, B.C. (2001): Yogurt as probiotic carrier food. Int. *Dairy J*., 11. 1-17.

Martin, J.H., Chou, K.M. (1992). Selection of Bifidobacteria for use as dietary adjuncts in cultured dairy foods: 1- tolerance to pH of yogurt. *Cultured Dairy Products Journal*, 27. 21-26.

Oliveira, R.P.S., Perego, P., Converti, A., Oliveira, M.N. (2009a) Growth and acidification performance of probiotics in pure culture and co-culture with Streptococcus thermophilus: The effect of inulin. *LWT-Food Sci. Technol*., 42. 1015-1021.

Oliveira, R.P.S., Perego, P., Converti, A., Oliveira, M.N. (2009b): Effect of inulin on growth and acidification performance of different probiotic bacteria in co-cultures and mixed culture with Streptococcus thermophilus. *J. Food Eng*., 9. 133-139.

Oliveira, R.P.S., Perego, P., Oliveira, M.N., Converti, A. (2011): Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk. *LWT-Food Sci. Technol*., 44. 520-523.

Özer, D., Akin, S., Özer, B. (2005): Effect of inulin and lactulose on survival of Lactobacillus acidophilus LA-5 and Bifidobacterium bifidum BB-02 in acidophilus-bifidus yoghurt. *Food Sci. Technol. Int*., 11. 19-24.

Pandey, S.M., Mishra H.N. (2015): Optimization of the prebiotic & probiotic concentration and incubation temperature for the preparation of synbiotic soy yoghurt using response surface methodology. *LWT-Food Sci. Technol*., 62. 458-467.

Paseephol, T., Sherkat, F. (2009): Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. *Journal of Functional Foods*, 1. 311-318.

Reilly, S.S., Gilliland, S.E. (1999): Bifidobacteirum longum survival during frozen and refrigerated storage as related to pH during growth. *J. Food Sci*., 64. 714-718.

Ramchandran, L., Shah N.P. (2010) Influence of addition of Raftiline HP on the growth, proteolytic, ACE- and α-glucosidase inhibitory activities of selected lactic acid bacteria and Bifidobacterium. *LWT-Food Sci. Technol*., 43. 146-152.

Roberfroid, M.B. (1999): Concepts in functional foods: the case of inulin and oligofructose. *J. Nutr*., 129. 1398S-1401S.

Roberfroid, M.B., van Loo, J.A.E., Gibson, G.R. (1998): The bifidogenic nature of chicory inulin and its hydrolysis products. *J. Nutr*., 128. 11-19.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J.M., Bressollier, P. (2013): An overview of the last advances in probiotic and prebiotic field. *LWT-Food Sci. Technol*., 50. 1-16.

Sanz, Y. (2007): Ecological and functional implications of the acid-adaptation ability of Bifidobacterium: A way of selecting improved probiotic strains. *Int. Dairy J*., 17. 1284-1289.

Su, P., Henriksson, A., Mitchell, H. (2007): Selected prebiotics support the growth of probiotic mono-culturesin vitro. *Anaerobe*, 13. 134-139.

Talwalkar, A.I., Kailasapathy, K.(2003): Effect of microencapsulation on oxygen toxicity in probiotic bacteria. Aust. *J. Dairy Technol*., 58. 36-39.

Talwalkar, A.I., Kailasapathy, K. (2004): The role of oxygen in the viability of probiotic bacteria with reference to L. acidophilus and Bifidobacterium spp. *Current Issues in Intestinal Microbiology*, 5. 1-8.

Zhao, X.H., Li, D. (2008): A new approach to eliminate stress for two probiotics with chemicals in vitro. Eur. Food Res. Technol., 277. 1569-1574.

Zhao, H., Zhang, L. (2006): Growth of probiotic bacteria in milk supplemented with protein hydrolysate. *China Dairy Ind*., 44. 16-18.

Zimeri, J.E., Kokini, J.L. (2003): Rheological properties of inulin waxy maize starch systems. *Carbohyd Polym*., 52. 67-85.

Food and Agriculture Organization of the United Nations, World Health Organization – FAO/WHO. (2001*)*: Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. Córdoba, Spain: Food and Agriculture Organization of the United Nations, World Health Organization.* 34 *p.*

Corresponding author (*levelezési cím*):

Éva Varga-Visi
Kaposvár University, Faculty of Agricultural and Environmental Sciences
H-7401 Kaposvár, P.O. Box 16.
Tel.: 36-82-505-800, Fax: 36-82-321-749
e-mail: vargane.eva@ke.hu