SYNBIOTIC PROPERTIES OF FUNCTIONAL FOOD ADDITIVE FROM JERUSALEM ARTICHOKE TUBERS IN REGARD TO LACTIC ACID BACTERIA STARTER CULTURES FOR FERMENTED FOODS

Pavels Semjonovs, Aleksejs Danilevics, Dagnija Upite, Ellena Kaminska, Elina Runca, Mara Marauska, Martins Bekers

University of Latvia, Institute of Microbiology and Biotechnology, Kronvalda boulv. 4, Riga LV 1010, Latvia, e-mail: psem@latnet.lv

Abstract
Since health benefits of lactic acid bacteria and bifidobacteria consumption have been recognized extensive studies on specific health effects and technology aspects have been reported. Fructans are generally proved prebiotics and potent supplements for synbiotic foods, nevertheless the interaction of innovative prebiotic substances, starter cultures and involved probiotic strains, as well as food matrices should be evaluated for each one combination under consideration. A technology for production of fructan-containing Jerusalem Artichoke (*Helianthus tuberosus*) Concentrate (JAC) has been developed. The predominance of fructooligosaccharides (DP 3–8) as well as various macro- and micronutrients in the composition of JAC makes them different from a number of commercial fructan sources and provides good prebiotic properties, being generally suggested for this range of fructans. Supplementation of milk and oat- hydrolysate medium with JAC and subsequent fermentation with different probiotic dairy starters resulted in substantial stimulation of bacterial growth (probiotics *B. lactis* and *L. acidophilus* as well as *L. bulgaricus*) and acidification rate. The strain-specific responses of two general yogurt cultures *L. bulgaricus* and *S. thermophilus*, as well as probiotic strains by addition of JAC should have been considered during design of prebiotic starters and conditions of fermentations process. JAC is suggested to be perspective prebiotic fructan-containing additive for fermented synbiotic milks or oat-hydrolysate based products.
Key words: prebiotic, probiotic, fermented, milk, fructan

Introduction
The tenet "Let food be thy medicine and medicine be thy food," espoused by Hippocrates nearly 2.500 years ago, is receiving renewed interest. In particular, there has been an explosion of consumer interest in the health enhancing role of specific foods or physiologically-active food components, so-called functional foods. Clearly, all foods are functional, as they provide taste, aroma, or nutritive value. Within the last two decades, however, the term functional as it applies to food has adopted a different connotation - that of providing an additional physiological benefit beyond that of meeting basic nutritional needs (Hasler, 1998).
As scientific and technological advances develop in the field of health and nutrition, more and more focus has been directed toward the emerging field of functional foods. Biologically active components in functional foods may impart health benefits or desirable physiological effects. Functional attributes of many traditional foods are being discovered, while new food products are being developed with beneficial components. Consumer interest in the relationship between diet and health has increased the demand for information and research about functional foods. Rapid advances in science and technology, increasing healthcare costs, changes in food laws affecting label and product claims, an aging population, and rising interest in attaining wellness through diet are among the factors supporting worldwide interest in functional foods. Credible scientific research indicates there are many clinically demonstrated and potential health benefits from food components. A large body of credible scientific research is needed to confirm the benefits of any particular food or component. For functional foods to deliver their potential public health benefits, consumers must have a clear understanding of and a strong confidence in the scientific criteria that are used to document health statements and claims. The scientific community continues to increase its understanding of the potential for functional foods and their role in health (IFIC, 2006).
Products obtained by lactic acid fermentation processes therefore are of special importance for functional foods since the synbiotic (probiotic & prebiotic) properties could be obtained by an adequate combination of lactic acid bacteria LAB or bifidobacteria (Schrezenmeir *et al.*, 2001), besides with prebiotic substances such as oligo- and polysaccharides of plant and

microbial origin (Semjonovs *et al.,* 2007a;b). The use of any prebiotic substance for the enrichment of fermented products provides its delivery into human gastrointestinal tract (GIT) and, hence, a stimulation of beneficial probiotic bacteria regardless of their origin (native GIT inhabitants or delivered by functional products). The overall prophylactic and therapeutic qualities of fermented functional foods substantially depend on the content of viable probiotic cells. Therefore it is necessary to achieve a high cell count for probiotic bacteria (e.g. *Bifidobacterium lactis*) at the stage of lactic acid fermentation of product by an addition of potent prebiotic substances (Semjonovs *et al.,* 2004; 2007a;b;2008). Fructose polymers and oligomers (Flamm *et al.,* 2001) as well as exopolysaccharides of LAB (Semjonovs *et al.,* 2008) should be considered as most appropriate additives for this purpose. However, bifidogenic properties of prebiotics, being well manifested in GIT, remain not so evident at the stage of fermentation of synbiotic product with probiotics containing starters, particularly against a high background of basic carbon source, e.g. milk lactose. Besides, it is necessary to evaluate possible growth responses of basic starter cultures (for instance, *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* for yogurt production) to the prebiotic additives during fermentation process since varied relations between them could substantially affect overall sensory properties and technological requirements.

The perennial vegetable plant *Helianthus tuberosus* (Jerusalem artichoke) which can be successfully cultivated in Latvia is a promissing plant as regards functional food constituents, as well as tubers and fresh tops can be used as fodder. The tuber flesh of this plant is a rich source for ß-2.1 fructooligo- and polysaccharides (e.g., inulin) (Bekers *et al.,* 2007b) acting as sweeteners that not affect blood sugar level after ingestion, as well as dietary fibre, blood cholesterol lowering agent, efficient prebiotics, stimulators of calcium absorption in the digestive tract etc. (Flamm *et al*., 2001).

The aim of this study is to assess the influence of Jerusalem artichoke concentrate (JAC) and other fructans sources on the growth of common constituent cultures of probiotic starters in milk and oat substrates and, thereby to evaluate the suitability of JAC for development of fermented synbiotic foods.

Materials and Methods

Probiotic strains *Bifidobacterium lactis* Bb12, *Lactobacillus acidophilus* La5, as well as milk cultures *Lactobacillus delbrueckii ssp. bulgaricus Lb12 (L. bulgaricus)* and *Streptococcus thermophilus* ST (Chr. Hansen, Denmark) were used. Two percent of overnight culture grown in the MRS-lactose medium (Semjonovs, *et al.,* 2007a) was used as an inoculum for fermentations of individual strains, as well as for following compositions of mixed probiotic starters: (A): *L. bulgaricus, S. thermophilus, B. lactis, L. acidophilus*; (B): *L. bulgaricus, S. thermophilus, B. lactis*; (C): *S. thermophilus. B. lactis. L. acidophilus).* The equal cell concentration for each constituent strain in starter compositions (A, B, C) was monitored by OD_{550} measurements of individual cell suspensions). Fermentations were performed in 250 ml flasks containing 150 ml either milk- or oat- media, at 37 ºC in the semianaerobic environment (BD BBL™ GasPak™ Anaerobic System Envelopes, USA). Viable lactic acid bacteria and bifidobacteria were enumerated by use of plate-count method in accordance with IDF 177A:1988. Titrable acidity (ºT) was determined by alkaline titration (0.1 N NaOH) of samples, using phenolphthalein as an indicator (ISO 750:1998). Skim milk medium was prepared from reconsituted (75 g l^{-1} tap water) skim milk powder (Valmieras piens, Latvia). The thermal and enzymatic treatments of commercial rolled oats and the preparation of oat medium were performed in accordance with LV 12304 (Bekers *et al.,* 1999). Each experiment was performed at least in triplicate and data are presented as averages, where Standard deviations did not exceed 10% of the mean.

Results and Discussion

A technology for production of Jerusalem artichoke concentrate has been developed (Bekers *et al.*, 2007a;b). Jerusalem artichoke concentrate powder besides a short chain inulin and fructooligosaccharides (up to 70% from the total carbohydrates) contains other valuable components: proteins, plant lipids, as well as K, Mg, Zn, Cr and several other macro- and microelements *(*Mullin *et al.*, 1994). The employed technology includes washing of tubers, chipping, and dehydration, roasting and grinding to obtain powder with 5–7% of water content. The chemical composition of JAC obtained in accordance with originally developed procedure *(*Bekers *et al*., 2007a*)* is (% dry mass): solids, 94.6; total carbohydrates, 63.7 (including fructans, 45.0; sucrose, 8.5; fructose, 3.4; glucose, 0.8; others 4.9); proteins, 17.1; lipids, 1.9; nucleic acids, 2.1. The taste properties of JAC can be regulated by activating exo-inulase, the enzyme found in tubers, as well as by regulating dehydratation and roasting temperatures. The taste of JAC slightly reminds the taste of rye bread or malt. JAC can be used as additive to confectionary, bread, fermented milks and other products. Fermented milk or cereal hydrolysate products are excellent carriers for development of probiotic bacteria *(*Bekers *et al.*, 1999; Semjonovs *et al.*, 2007b; 2008) and can be enhanced by fructans or other prebiotic substances *(*Semjonovs *et al.*, 2004; 2007a;b; 2008*)*. The dried concentrate of Jerusalem artichoke (*Helianthus tuberosus*) tubers containing 45–50% (d. w.) of fructans together with other biologically active macro- and micronutrients differed from commercial fructan sources in respect to both chemical (Bekers *et al.*, 2007b) and prebiotic properties (Table 1 and 2). The HPLC analysis of fructans from Jerusalem artichoke concentrate (JAC) displayed a prevalence of fructan oligosaccharides (FOS) of varied degree of polymerization (DP 3 – 8) as compared to the relative contribution of inulin (DP ~ 31), disaccharides and monosaccharides.

Table 1

The influence of fructan supplements (2g $100g^{-1}$) on the growth of probiotics *Bifidobacterium lactis* Bb12 and *Lactobacillus acidophilus* La5, as well as general yogurt cultures *Lactobacillus bulgaricus* Lb5 and *Streptococcus thermophilus* ST in milk, after 12 h fermentation

Cultures	Fructan-containing supplements						
	Milk (Control)	+Inulin[a]	+FOS[b]	+Raftiline[c]	+Raftilose[d]	+JAC	+ Levan[e]
	Cell-count, cfu ml^{-1}						
B. lactis	$2.03x10^7$	$2.08x10^7$	$2.82x10^7$	$2.87x10^7$	$2.89x10^7$	$3.04x10^7$	$3.12x10^7$
L. acidophilus	$5.27x10^7$	$6.26x10^7$	$6.98x10^7$	$8.19x10^7$	$6.42x10^7$	$8.72x10^7$	$6.24x10^7$
L. bulgaricus	$6.20x10^8$	nd	$1.272x10^9$	nd	nd	$1.31x10^9$	nd
S. thermophilus	$9.37x10^8$	nd	$9.53x10^8$	nd	nd	$9.68x10^8$	nd

a) Inulin (inulin of analytical purity; Sigma- Aldrich, Germany);
b) FOS (NutrafloraFOS®- fructooligosaccharides; Twinlab, USA);
c) Raftiline ST ® (food-grade inulin, Orafti, Belgium);
d) Raftilose L60/75 ® (food-grade fructooligosaccharides, Orafti, Belgium);
e) Levan - high molecular weight (2000 kD) ß-2,6 polyfructan, produced by *Zymomonas mobilis*

The observed fructan composition of JAC therefore confirms the occurrence of possible shift towards short-chain fructans caused by thermal treatment and/or action of endo-inulinases during a processing of native Jerusalem artichoke tubers which contain about 50% of inulin (DP 11–30) (Semjonovs *et al.*, 2007b).

Table 2

The influence of JAC (2.0 %) on the development of titrable acidity during fermentation of milk by various starters

Starter composition	Medium	Time, h		
		3	6	9
		Titrable acidity, ºT		
A *B. lactis* *L. bulgaricus* *S. thermophilus*	Milk (Control)	24.0	34.2	42.0
	+ JAC	36.6	68.0	85.0
B *B. lactis* *L. acidophilus* *L. bulgaricus* *S. thermophilus*	Milk (Control)	20.0	32.0	37.5
	+ JAC	34.4	71.3	100
C (ABT-type) *B. lactis* *L. acidophilus* *S. thermophilus*	Milk (Control)	22.4	32.0	42.0
	+ JAC	33.4	80.0	101.0

The predominance of oligosaccharides (DP 3–8) in the composition of JAC obviously makes them different from a number of other commercial components and could provide good prebiotic properties, being generally suggested for this range of fructan oligomers (Flamm *et al.* 2001).

Table 3

The total cell-count and titrable acidity in oat-medium after 8 h fermentation by various starters

	A *B. lactis, L. bulgaricus, S. thermophilus*		**B** *B. lactis, L. Acidophilus, L. Bulgaricus, S. thermophilus*		**C (ABT-type)** *B. lactis, L. acidophilus, S. thermophilus*	
	cfu mg^{-1}	ºT	cfu mg^{-1}	ºT	cfu mg^{-1}	ºT
Oat medium (Control)	$1.12x10^{9}$	88	$3.96x10^{9}$	94	$3.66x10^{8}$	82
+ JAC, 2 %	$7.31x10^{9}$	99	$4.74x10^{10}$	108	$5.83x10^{9}$	97

Supplementation of milk with JAC fermented by three different probiotic starters *significantly* increased the yield of bacterial biomass and acidification rate. Besides, increased cell-counts of probiotics *B. lactis* and *L. acidophilus*, as well as *L. bulgaricus* were observed. A relatively wide choice of universally known prebiotic fructan supplements were compared to JAC during growth of *B. lactis* and *L. acidophilus* in milk media. The effect of JAC substantially (7–38%) exceeded those observed for commercial food-grade fructan sources such as Nutraflora FOS®, Raftiline ST® and Raftilose L60/75 ® in respect of *L. acidophilus.* Relative growth responses of *B. lactis* also indicated slight (5–8%) but significant advantages of JAC in comparison with other fructan additives. The growth of *S. thermophilus* was only negligible (1.02–1.08 times) affected by addition of JAC.

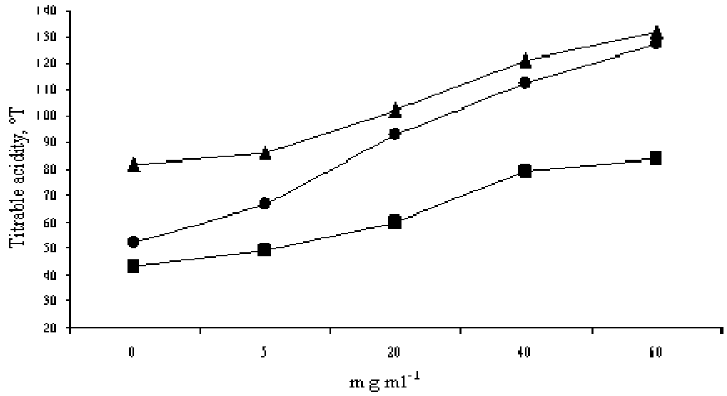

Figure 1. An increase of titrable acidity upon the various concentration of JAC in milk fermented by *L. bulgaricus* (▲), *L. acidophilus* (●) and *B. lactis* (■), after 12 h fermentation

The influence of JAC on the cell count of individual cultures involved as constituents in different starters during fermentation of milk or oat-hydrolysates (Table 2 and 3) showed the prebiotic properties upon *B. lactis* and *L. acidophilus*. The distinctions (Figure 1; Table 1) in responses of two general yogurt cultures *L. bulgaricus* and *S. thermophilus* to the addition of JAC, as well as another fructans (Table 1) could be of significance to design chemical, rheological and organoleptic properties of yogurts.

Conclusions

The above results suggest the good properties for application of JAC in the production of synbiotic oat- or milk-based fermented foods. The comparative evaluation of novel fructan-containing functional additive from Jerusalem Artichoke tubers showed very distinctive responses of probiotic cultures *B. lactis* and *L. acidophilus*, as well as two general yogurt cultures *L. bulgaricus* and S. *thermophilus* on the addition of fructan source to the food substrate under fermentation. An application of JAC for enhancement of fermented milks obviously requires an employment of certain food additives (starch, pectin etc.) for stabilisation of JAC particles and thus, an evaluation of possibly interacting stabilizer effects in regard to employed strains, as well as an overall accommodation of any particular fermentation process.The functional qualities of synbiotic fermented foods can be substantially improved due to enhanced cell-count of viable probiotics caused by addition of potent prebiotic substances (e.g. JAC or other fructan sources) to food substrates under fermentation. The prebiotic efficacy of JAC supplements shown to be of exceeding or equal worth as compared with other commercial fructan sources and, has been significantly manifested in fermentations performed by both, individual strains and starters.

Acknowledgments

This study was supported by the European Social Foundation. Project ESSS 2004/3. Contracts' Nr. 2004/0001/VPD1/ESF/PIAA/04/NP/3.2.3.1/0001/0001/0063

References

1. Hastler, C. M. (1998) A new look at an ancient concept. *Chem Industry*, 2:84–89.
2. IFIC International Food Information Council Foundation // Functional Foods. 2006. Source: http.://www.ific.org; resource used on 30.01.2008.
3. Schrezenmeir, J., de Vrese, M. (2001) Probiotics, prebiotics, and synbiotics-approaching a definition. *Am J Clin Nutr*, 73:361S–4S.
4. Semjonovs, P., Zikmanis, P. (2007a) An Influence of Levan on the Fermentation of Milk by a Probiotic ABT-Type Starter. *J Food Technol*, 5: pp. 123–130.
5. Flamm, G., Glinsmann, W., Kritchevsky, D., Prosky, L., Roberfroid, M. (2001) Inulin and oligofructose as dietary fiber: a review of the evidence. *Crit Rev Food Sci Nutrit* 41(5): pp. 353–362.

6. Semjonovs, P., Jasko, J., Auzina L., Zikmanis, P. (2008) The use of exopolysaccharide-producing cultures of lactic acid bacteria to improve the functional value of fermented foods. *J Food Technol*, 6(2): pp.101–109.
7. Bekers, M., Marauska, M., Laukevics, J., Grube, M., Karklina, D, Viesturs, U., Vigants, A. (1999) Polifunkcionālu pārtikas produktu ieguves paņēmiens no graudu izejvielām. Patent application: *LV12304.*
8. Bekers, M., Viesturs, U., Danilevics, A., Upite, D., Kaminska, E., Marauska, M., Grube, M., Linde, R. (2007a). Method of production and use of fructans and fructose containing products from inulin caontaining raw materiāls. Patent application: *LV13539.*
9. Bekers, M., Grube, M., Upite, D., Kaminska, E., Linde, R., Scherbaka, R., Danilevics, A. (2007b) Carbohydrates in Jerusalem artichoke powder suspension. *Nutr Food Sci*, 37(1): pp. 42–49.
10. Semjonovs, P., Zikmanis, P., Bekers, M. (2007b) An influence of fructan containing concentrate from jerusalem artichoke tubers on the development of probiotic dairy starters on milk and oat-based substrates. *Food Biotechnol*, 21 (4): pp. 347–363.
11. Semjonovs, P., Marauska, M., Linde, R., Kozlinskis, E., Zikmanis, P., Bekers, M. (2004) *Influence of fructans on the fermentation performance of Bifidobacteria containing starters.* International Conference (Moscow, Russia), Probiotics, prebiotics, synbiotics and functional food products – up to day and future. pp. 115–116.
12. Mullin, W. J., Modler, H. W., Farnworth, E. R., Payne, A. (1994). The macronutrient content of fractions from Jerusalem artichoke tubers (*Helianthus tuberosus*). *Food Chem*. 51(3): pp. 263–269.