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Prebiotic effectiveness of inulin extracted from jerusalem artichoke (*helianthus tuberosus*)

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ABSTRACT

The prebiotic potential of fructooligosaccharides derived from native Jerusalem artichoke tubers (JA-Fr) was assessed by monitoring in vitro effects of JA-Fr on bacterial growth. Results showed the greater survivability of beneficial bacteria (*lactobacillus acidophilus*) in cultures media containing JA-Fr in comparison with HP-inulin (a high molecular-weight fraction of chicory-derived inulin). Both JA-Fr and HP-inulin led to a significant increase in bacterial population compared to the control ($P<0.05$), and the efficacy was proportional to the degree of polymerization (*DP*). The pH decreased during the *lactobacilli* activity and no significant differences were observed in the pH with both inulin-type fructans. In addition, in vitro evaluation of specific growth rates of *Escherichia coli* showed that the growth of these bacteria were influenced by the *DP* and concentration of fructans used in the media.

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KEYWORDS

Jerusalem artichoke tuber;
Fructan;
Prebiotics;
Inulin;
Probiotics.

INTRODUCTION

The microflora within the human gastrointestinal tract plays an important role in the health and quality of life of the customers, and many attempts have been made to modify the intestinal microbial balance[1].

Lactic acid bacteria such as *Lactobacilli* and *Bifidobacteria* are well-known probiotics isolated from normal human and animal gastrointestinal tract (*GIT*). When used in adequate amounts in diet, they can survive the passage through the digestive tract, adhere to intestinal cells and modify the metabolic activities in the body[2, 3].

Beneficial mechanisms of probiotics action include: modulation of immune system[4], production of bacteriotoxins[5], synthesis of vitamins (such as *K* and *B*), stabilization of barrier functions and enhance the calcium and other mineral absorption on the gut. Different studies have also demonstrated positive effects of probiotic bacteria on bowel pH, intestinal regularity and the colonization resistance against pathogens[3, 6].

Prebiotics are selectively fermented ingredients

that allow beneficial changes, both in the activity and/or composition of the gut microorganisms and affect host well-being. They promote the growth of healthy bacterial populations and reducing pathogens counts[7].

The most documented prebiotics are fructooligosaccharides (*FOS*, Oligofructose and inulin) which are composed of *β(2-1)* linked fructosyl units with or without a terminal D-glucose at the reducing end. They have different degree of polymerization (*DP*) and may originate naturally as native components in many plants or derive through biochemical/enzymatic techniques[8-10].

The two plant species currently used in commercial production of prebiotics are Jerusalem artichoke (*Helianthus tuberosus*) and Chicory (*Cichorium intybus*), belong to *Compositae* family[11, 12].

The tuber of Jerusalem artichoke is traditionally cultivated as food and animal feed[13]. It contains nearly 13-18% carbohydrates, of which about 80% are inulin type fructans, 10-13% sucrose, 3.5-5% reducing sugars, 10-17% proteins[14] and 0.8-0.9% important minerals including *K, Ca, P, Fe, Zn, Mg, Na, Cu* and *Mn*[15, 16].

The *DP* of fructans of Jerusalem artichoke tubers is rather low[17] and mainly depends on the plant source, variety, climate conditions and date of harvest[18, 19]. In this study, we investigated the prebiotic potentials of fructooligosaccharides extracted from native Jerusalem artichoke tubers on the survivability and fermentation activity of an in vitro cultured probiotic bacteria and compared the results with prebiotic effects of HP-inulin. In addition, we evaluated the growth of *Escherichia coli* in the cultures containing these prebiotics.

MATERIALS AND METHODS

Bacteria and media preparation

The commercially available probiotic strain was *Lactobacillus acidophilus La5* which obtained from Chr. Hansen (Horsholm, Denmark). *Escherichia coli PTCC 1330* was obtained from Persian Type Culture Collection. The standard prebiotic of HP-inulin purchased from Orafti (Tienen, Belgium). The fructooligosaccharides of Jerusalem artichoke tubers (JA-Fr) was extracted according to Milani et al.[20]. Growth media were MRS broth (DeMan-Rogosa-Sharp Broth) and TSB broth (Trypticase Soy Broth), purchased from Merck (Darmstadt, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany). The fructooligosaccharides free media were used as the control and the basal media. The inoculums were prepared from the standard strains stored in glycerol 12% at –70°C (degree centigrade) using basal media. The carbohydrates (JA-Fr and HP-inulin) were filter-sterilized and added to the basal MRS broth and basal TSB broth to give final concentrations of 0.5%, 1%, 2% and 3% (w/v).

Growth of *Lactobacillus acidophilus La5* and *Escherichia coli PTCC 1330* in presence of prebiotics

To study the effects of JA-Fr on the growth and survivability of *lactobacillus acidophilus* and *Escherichia coli* strains, the bacteria were cultivated overnight in the appropriated basal medium at 37ºC. The activated cultures centrifuged for 15 min with 2500×g at 4ºC (sigma centrifuge model 2-16p), then the precipitate was washed twice with PBS (0.1 M phosphate buffer pH 7.4, 0.9% saline), and the final pellet was suspended in PBS and diluted to about 10v cells/mL for *L. acidophilus* and 10y -10¹p cells/ml (milliliter) for *E. coli*. The bacterial suspensions were inoculated at 1% (v/v) in to different testing media containing fructans. Then the cultures were incubated at 37ºC for 24 hours. The turbidity at 620 nm of each culture was determined every 4 hours for up to 24 hours by subtracting A_{620} values of bacterial free medium from each test media. This was repeated two times.

Determination of pH changes

pH value was measured using Inolab pH meter model WTW (Inolab).

Growth kinetic parameters

Specific growth rate (μ) was calculated for each

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microorganism during its exponential growth phase by the equation:

$$\mu = (Lnx - Lnx0) / (t - t0)$$

Where x and x0 are absorbance measured at time t and *t0*, respectively.

The generation time (*tg*) was calculated for each culture from the corresponding value of (μ) by the equation:

$$tg = Ln2 / \mu$$

Statistical analysis

The results obtained were statistically analyzed using MINITAB 14 and MSTATC software and significant differences between groups were determined by the Duncan's multiple range test. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Bacterial growth study

In order to investigate the effects of JA-Fr on

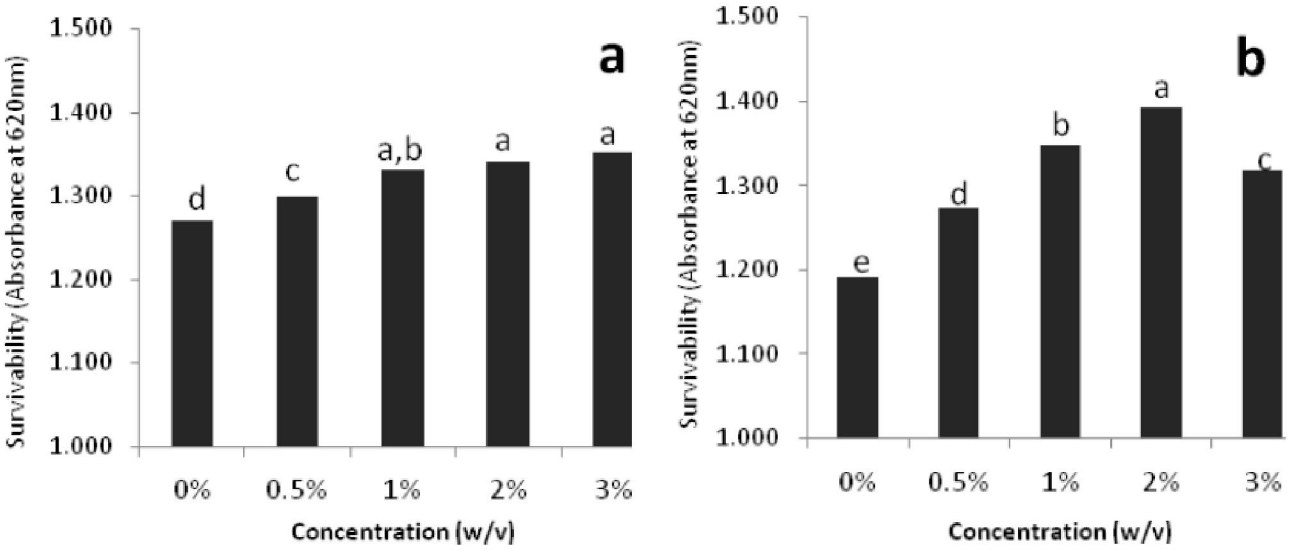

Figure 1 : Effects of JA-Fr concentrations on the viability of *L. acidophilus* (A) and *E. coli* (b). Different letters mean statistically significant difference among the values of the same parameter, according to Duncan test (P < 0.05)

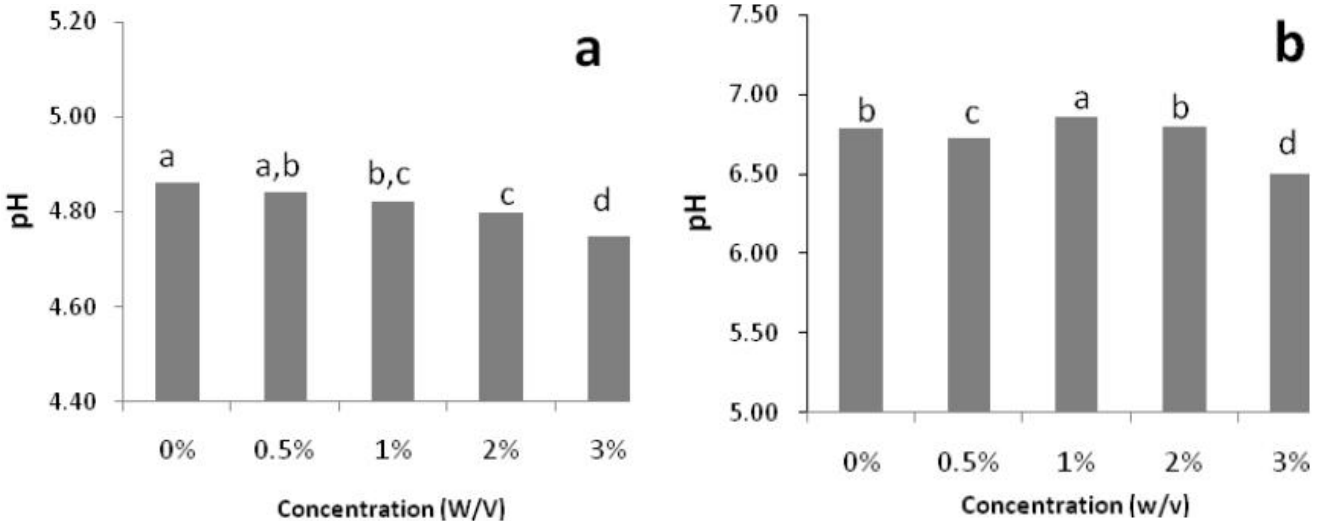

Figure 2 : Effects of JA-Fr concentrations on pH changes of media inoculated with *L. acidophilus* (a) and *E. coli* (b). Different letters mean statistically significant difference among the values of the same parameter, according to Duncan test (P < 0.05)

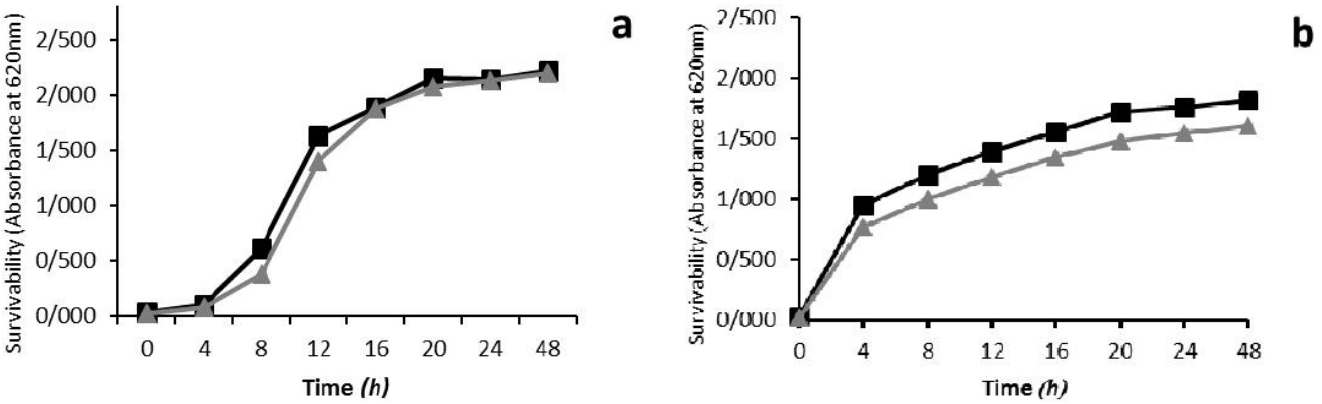

Figure 3 : Growth kinetics of *L. acidophilus* (a) and *E. coli* (b) in appropriate medium enriched with JA-Fr (■) and HP-inulin (▲) at 3% (w/v) during 48-h incubation at 37 °C

TABLE 1 Comparison of bacterial specific growth growth rates (μ) in the presence of different fructans

	Control	JA-Fr	HP-inulin
Lactobacillus acidophilus	0.397±0.002	0.458±0.005*	0.380±0.005
Escherichia coli	0.763±0.003	0.885±0.004*	0.772±0.007

All values for μ (h^{-1}) are means from duplicate determination ± SD. In the same row, significant differences at P<0.05 confidence intervals (according to Duncan test) are shown as *

the growth of *L. acidophilus* and *E. coli*, the organisms were cultured in the appropriate media supplemented with different concentrations of JA-Fr at 37 ºC for 24 h (hours). *Lactobacillus acidophilus La 5* and *Escherichia coli PTCC 1330* are the strains associated with human or animal digestive systems. Results showed that both JA-Fr and HP-inulin were fermented by the microbial flora (Figure 4). As shown in Figure 1(a), JA-Fr had the potential to stimulate the growth of *L. acidophilus* (*La 5*). The amount of cell growth increased as the concentration of JA-Fr rose to 3% (w/v). Supplementation with JA-Fr was found to have significantly better effect on stimulating the growth of the bacteria compared with that without JA-Fr ($p<0.05$).

Growth curve of *L. acidophilus* showed that the bacteria grew rapidly after about 4h, with maximum growth observed at about 24h, and then it reached to the plateau phase (Figure 3 (a)).

Specific rates of growth were determined for the media supplemented with 3% (w/v) JA-Fr compared to the control and HP-inulin (TABLE 1). JA-Fr caused greater in vitro growth rate of lactobacilli than did the control and HP-inulin.

The results showed that the growth behavior of bacteria in the presence of 3% HP-inulin was similar with the control. In the case of the lactobacilli, as reported in other studies, growth of strains on fructooligosaccharides appears to be species or strain specific[21, 22]. With fewer studies reported concerning fructan consumption, the preference for short-chain fructooligosaccharides appears to be stricter, only using compounds of *DP* from 2 to 3[22-25]. Wichienchot et al. showed that oligosaccharides extracted from white-flesh dragon fruit used as a carbon source, significantly stimulated the growth

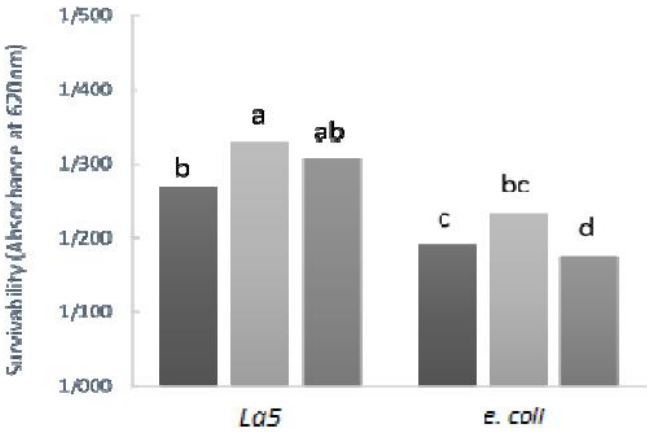

Figure 4 : Comparative growth of *L. acidophilus* and *E. coli* in the presence of JA-Fr (light grey) HP-inulin (dark grey), and control (black) after incubation at 37 °C for 48 hours. The data represent the results of a duplicate experiment

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of *L. delbrueckii*[26]. In a consistent findings, using in vivo studies, Gibson et al. reported that numbers of lactobacilli were not affected by oligofructose (*DP* 2-6), but inulin increased lactobacilli counts, although not significantly (P = 0.075)[27].

In vitro experiments on the comparative fermentation of HP-inulin and JA-Fr in the cultures inoculated with *E. coli* showed that growth promotion of *E. coli* by JA-Fr is dose dependently over the range 0.5% to 3% as evidenced by increased turbidity of the bacteria suspensions (Figure 1(b)), indicating that *E. coli* grew faster in the presence of these carbohydrates. Figure 3(b) shows the growth curves of *E. coli* strain cultured with both carbon sources. HP-inulin was found to be less effective on the viability of *E. coli*. The doubling time (*tg*) of the strain grown in the presence of JA-Fr, HP-inulin and the control medium are compared in TABLE 2. Doubling time was used as a measure of the efficacy of various carbon sources in modulating growth rate. *tg* in the medium containing JA-Fr was minimal.

In general, the ability of coliforms to utilize prebiotic oligosaccharides has been contradictory. Several studies have reported that FOS can support growth of *E. coli*, *Enterobacter* and *Salmonella*[28, 29]. In contrast, others have indicated no growth of *E. coli*[21, 30-32].

Lopez-Molina et al. studied the utilization of chicory and Artichoke inulin (different *DP*) in mixed cultures of colonic bacteria and showed that growth of *Escherichia coli* and total anaerobes was slower but longer-lasting in the presence of both inulin compared to the control with glucose[33]. Van Laere et al. reported that arabinooligosaccharides could support the growth of *E. coli* but FOS could not[34].

Our findings were implying that the degree of polymerization of fructans was an important factor that decides the accessibility of fructans to the bacteria. According to Biedrzycka and Bielecka, susceptibility of saccharides to fermentation mainly depends on water solubility, chemical structure, degree of polymerization, chain length, branched or linear structure and composition of monomer units[35]. Roberfroid et al. reported in vitro fermentation of inulin by human fecal bacteria, molecules with *DP*>10 were fermented on the average half as quickly as molecules with *DP*<10[36]. The degree of polymerization of fructans from *Helianthus tuberosus* tubers is rather low[17] in comparison with HP-inulin and mainly depends on the variety, climate conditions and time of harvest[18, 19].

The pH of bacterial extract was recorded for the strains grown with the various carbohydrates. In the case of *L. acidophilus*, as shown in Figure 2(b),

TABLE 2 : Doubling times (*tg*) of the strains in different media

	Control	JA-Fr	HP-inulin
Lactobacillus acidophilus	1.746±0.002	1.513±0.005*	1.824±0.005
Escherichia coli	0.908±0.003	0.783±0.004*	0.898±0.007

All values for tg (h) are means from duplicate determination ± SD. In the same row, significant differences at P < 0.05 confidence intervals (according to Duncan test) are shown as *

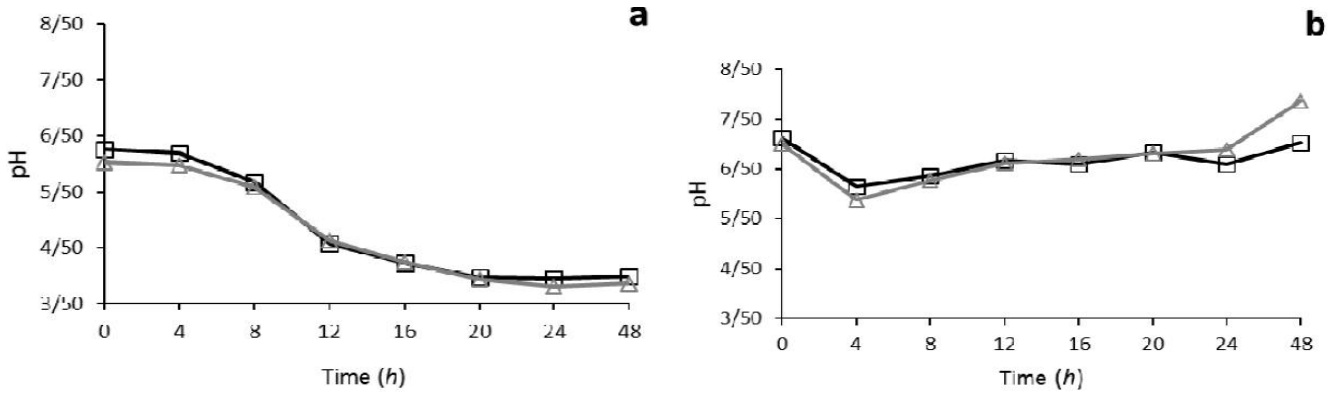

Figure 5 : Changes in pH of media inoculated with *L. acidophilus* (a) and *E. coli* (b) in the appropriate medium enriched with JA-Fr (□) and HP-inulin (△) at 3% (w/v) during 48 h incubation at 37 °C

a pH decrease correlates with the population growth during the incubation period, indicating the production of acetic and lactic acids[37]. The pH continued to decline in a similar way for the control media and in the presence of both JA-Fr and HP-inulin. After 24 hours of incubation, the pH dropped to 3.98 in JA-Fr-containing media, and to 3.86 in media with HP-inulin (Figure 5(a)). The differences of pH values between two fructans were significant ($p<0.05$). The lower pH is believed to have additional effects in body because the production of these acids reduces intestinal pH and restricts or prohibits the growth of many pathogen and putrefactive bacteria. Also it increases mineral uptake[38].

Figure 2(b) demonstrates the changes in TSB medium pH which occur during 24-h fermentation. pH has similar behavior in both media containing JA-Fr and HP-inulin. It was approximately 7.0 prior to inoculation, then there was a continued fall in medium pH over the first 4h and at this time, it began to rise (Figure 5(b)). This is probably due to *E. coli* growth and metabolism, especially the deamination of amino acids[39], since casein is a principal nutrient in TSP medium. The differences between the pH values of two media were significant ($p<0.05$).

CONCLUSION

Knowledge of the fermentative capacity of probiotic species can assist in both understanding of the effects that different non-digestible carbohydrates have on population dynamics in the intestinal microbiota and mechanisms of polysaccharide fermentation in the human colon. Results of current study demonstrated that improvement of growth, activity, and viability of *Lactobacillus acidophilus* and *Escherichia coli* in the media are dependent on the carbon source and concentration. Jerusalem artichoke fructooligosaccharides can affect probiotic survival during a 24-hour incubation period in, *in vitro* conditions and provide the greater stability and acid production to *L. acidophilus* (*La5*); however, further studies are needed with other probiotic strains used in human dietary. In addition, in vivo health benefits of native Jerusalem artichoke fructooligosaccharides still need to be investigated.

REFERENCE

[1] A.S.Neish; Gastroenterol., **136**, 65-80 **(2009)**.
[2] S.Parvez, K.A.Malik, S.AhKang, H.Y.Kim; Appl.Microbiol., **100**, 1171–1185 **(2006)**.
[3] T.Iannitti, B.Palmieri; Clinical.Nutr., **29**, 701-725 **(2010)**.
[4] F.Ibrahim, S.Ruvio, L.Granlund, S.Salminen, M.Viitanen, A.C.Ouwehand; FEMS.Immunol.Med.Microbiol., **59**, 53–59 **(2010)**.
[5] L.J.Fooks, R.Fuller, G.R.Gibson; Int.Dairy.J., **9**, 53–61 **(1999)**.
[6] M.J.Hopkins, G.T.Macfarlane; Appl.Environ.Microbiol., **69**, 1920–1927 **(2003)**.
[7] M.B.Roberfroid; Defining functional foods, in G.R.Gibson, C.M.Williams, Eds. 'Functional foods: concept to product', Chap 1, CRC Press, Boca Raton, 9–27 **(2000)**.
[8] O.Banuelos, L.Fernández, J.M.Corral, M.Valdivieso-Ugarte, J.L.Adrio, J.Velasco; Anaerobe., **14**, 184-189 **(2008)**.
[9] J.Van Loo, A.Franck, M.Roberfroid; Br.J.Nutr., **82**, 329 **(1999)**.
[10] T.Nakakuki; Pure.Appl.Chem., **74(7)**, 1245-1251 **(2002)**.
[11] G.Leroy, J.F.Grongnet, S.Mabeau, D.Le-Corre, C.Baty-Julien; J.Sci.Food.Agric., **90**, 1203–1209 **(2010)**.
[12] N.Kaur, A.K.Gupta; J.Biosci., **27**, 703–714 **(2002)**.
[13] M.Baldini, F.Danuso, M.Turi, G.P.Vannozzi; Indust.Crop.Prod., **19**, 25-40 **(2004)**.
[14] G.Patkai, J.Barta; 'Nutritive value of different Jerusalem artichoke varieties', Abstracts 9th Seminar on Inulin, Budapest, 9-10 **(2002)**.
[15] G.Nemeth, Z.Izsaki; Cereal.Res.Commun., **34**, 597-600 **(2006)**.
[16] J.Barta, G.Patkai; Acta Aliment., **36**, 257–267 **(2007)**.
[17] A.Bohm, I.Kaiser, A.Trebstein, T.Henle; Eur.Food.Res.Technol., **220**, 466-71 **(2005)**.
[18] T.Paseephol, D.Small, F.Sherkat; Food.Chem., **104**, 73–80 **(2007)**.
[19] M.Bekers, M.Grube, D.Upite, E.Kaminiska, R.Linde, R.Scherbaka, A.Danilevich; Nutr.food.sci., **37(1)**, 42-49 **(2007)**.
[20] E.Milani, H.Pourazarang, R.Kadkhodaee, H.Vakilian, S.Vatankhah; Iranian.Food.Sci.Technol. Res., **6(2)**, 113-120 **(2010)**.

FULL PAPER

[21] P.Monsan, F.Paul; FEMS.Microbiol.Rev., **16**, 187–192 **(1995)**.

[22] H.Kaplan, R.W.Hutkins; Appl.Environ.Microbiol., **66**, 2682–2684 **(2000)**.

[23] D.Ozer, S.Akin, B.Ozer; Food.Sci.Tech.Int., **11(1)**, 019–6 **(2005)**.

[24] V.Rosseau, J.P.Lepargneur, C.Roques, M.Remaud-Simeon, F.Paul; Anaerobe., **11**, 145–53 **(2005)**.

[25] D.M.A.Saulnier, D.Molenaar, W.M.De Vos, G.R.Gibson, S.Kolida; Appl.Environ.Microbiol., **73**, 1753–65 **(2007)**.

[26] S.Wichienchot, M.Jatupornpipat, R.A.Rastall; Food.Chem., **120**, 850–857 **(2010)**.

[27] G.R.Gibson, E.R.Beatty, X.Wang, J.H.Cummings; Gastroenterol., **108**, 975-982 **(1995)**.

[28] X.Wang, G.R.Gibson; J.Appl.Bacteriol., **75**, 373–380 **(1993)**.

[29] R.Hartemink, F.M.Rombouts; Gas formation from oligosaccharides by the intestinal microflora, in G.Boehm Ed. 'International Symposium on Pre and Probiotics', Wageningen: Wageningen Graduate School, 57–66 **(1997)**.

[30] H.Hidaka, T.Eida, T.Takizawa, T.Tokunaga, Y.Tashiro; Bifidobacteria.Microflora., **5**, 37–50 **(1986)**.

[31] T.Mitsuoka, H.Hidaka, T.Eida; Die.Nahrung., **31**, 427–436 **(1987)**.

[32] J.S.Bailey, L.C.Blankenship, N.A.Cox; Poult.Sci., **70**, 2433-2438 **(1991)**.

[33] D.Lopez-Molina, M.D.Navarro-Martinez, F.Rojas-Melgarejo, A.N.Hiner, S.Chazarra, J.N.Rodriguez-Lopez; Phytochem., **66**, 1476–1484 **(2005)**.

[34] M.J.Van Laere, R.Hartemink, M.Bosveld, H.A.Schols, A.G.Voragen; J.Agric.Food.Chem., **48**, 1644-1652 **(2000)**.

[35] E.Biedrzycka, M.Bielecka; Trends.Food.Sci.Technol., **15**, 170–5 **(2004)**.

[36] M.B.Roberfroid, J.A.E.Van Loo, G.R.Gibson; J.Nutr., **128**, 11-19 **(1998)**.

[37] V.Scardovi; 'Genus Bifidobacterium', in Bergey's Manualof Systematic Bacteriology 2, Williams & Wilkins, New York, 1418–1434 **(1986)**.

[38] L.Baffoni, F.Gaggìa, D.D.Gioia, B.Biavati; Ann.Microbiol., **62(1)**, 15–30 **(2012)**.

[39] J.E.Rosenbla1t, F.Schoenknechtand; Antimicrob.agents.chemother., 433-440 **(1972)**.