In vitro prebiotic activity of inulin-rich carbohydrates extracted from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers at different storage times by *Lactobacillus paracasei*

Irene A. Rubel [a,b,*], Ethel E. Pérez [a], Diego B. Genovese [a], Guillermo D. Manrique [b]

[a] *Planta Piloto de Ingeniería Química, Universidad Nacional del Sur, Consejo Nacional de Investigaciones Científicas y Técnicas, Camino La Carrindanga Km 7, B8000 Bahía Blanca, Argentina*
[b] *Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad Nacional del Centro de la Provincia de Buenos Aires, Av. del Valle 5737, B7400JWI Olavarría, Argentina*

ABSTRACT

In the present work the *in vitro* prebiotic activity of inulin-rich carbohydrates using *Lactobacillus paracasei* as probiotic microorganism was determined. With this purpose, inulin-rich carbohydrates samples from Jerusalem artichoke tubers stored at 4–5 °C during different times along an overall period of 8 months were extracted. Extraction yield, inulin content and average polymerization degree of inulin-rich carbohydrates samples were also determined. For all samples, extraction yield and inulin content decreased with storage time. In particular, inulin content exhibited values within the range of commercial food grade inulin (≥85%). Average polymerization degree values decreased with storage time ranging from 12.1 to 8.3. The *in vitro* prebiotic activity of inulin-rich carbohydrates samples was assessed using a prebiotic activity score which considered the growing ratio relative to glucose of *L. paracasei* as well as of the enteric bacteria *Escherichia coli* when such carbohydrates were used as C source. Inulin-rich carbohydrates extracted from tubers cold-stored during 4 months showed the highest prebiotic activity score, even higher than the corresponding to a commercial food grade inulin, indicating that this sample should be used as a prebiotic food ingredient, representing a potential alternative to the available commercial inulin obtained from chicory roots.

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1. Introduction

Inulin is a linear fructan, a plant reserve polysaccharide constituted by fructose molecules linked by β(2→1) bonds, with a terminal glucose unit linked by an α (1→2) bond (Kays & Nottingham, 2007). Its degree of polymerization (DP) typically ranges from 2 to 60 units, with number average values (DP_n) of 10–12 units (Madrigal & Sangronis, 2007). The DP of inulin depends on many factors, such as plant source, climate and growing conditions, harvesting maturity, and storage time after harvest (Saengthongpinit & Sajjaanantakul, 2005). Fructans with lower DP_n values ($DP_n < 10$) are known as oligofructose or fructooligosaccharides (FOS). The DP is an important property of fructans since it affects their functionality.

Inulin, as a naturally occurring storage carbohydrate, is present in more than 36,000 plant species (Kalyani Nair et al., 2010; Madrigal & Sangronis, 2007). The three species currently used by the industry to produce inulin are Jerusalem artichoke (*Helianthus tuberosus* L.), dahlia (*Dahlia* spp.) and chicory (*Cichorium intybus*). At present, the source most widely exploited for industrial obtaining of inulin and FOS is, by

far, roots of chicory. Jerusalem artichoke was the plant source selected for this study, due to its interesting agronomic and industrial potential in many parts of the world (Rébora, 2008). The inulin content of Jerusalem artichoke tuber ranges from 7 to 30% of fresh weight (around 50% of dry weight) (Kays & Nottingham, 2007).

Inulin is used as a functional food ingredient that offers a unique combination of interesting nutritional properties and important technological benefits (De Gennaro et al., 2000; Franck & De Leenheer, 2005; Wang, 2009; Pinheirode de Souza Oliveira, Perego, Nogueira de Oliveira & Converti, 2012). Because of its textural, organoleptic, and nutritional properties, inulin is used in the food industry as a foam/emulsion stabilizer, and fat/sugar replacer, while it promotes further health benefits (Choque Delgado et al., 2010; Mantzouridou & Vassilios Kiosseoglou, 2012). In this regard, its property of soluble fiber with prebiotic capacity is one of the most relevant characteristics. Fructans are resistant to digestion and absorption in the human small intestine, and reach the colon, where they are selectively fermented by beneficial bacteria, such as *Lactobacillus* and *Bifidobacteria*. These microorganisms are known as probiotics, and have been reported to have health-promoting properties such as inhibition of exogenous pathogens (*e.g. Escherichia coli* and *Clostridium* spp.), and potentially treat colonic dysfunctions (Adebola et al., 2013; Kalyani Nair et al., 2010; Madrigal & Sangronis, 2007). López-Molina et al. (2005) reported that the relative rate of fermentation *in vitro* was similar for inulins from chicory and artichoke by

Bifidobacterium spp., *Bacteroides fragilis*, *Lactobacillus* spp., *E. coli* and total anaerobes.

To date, most studies are focused on utilization of short-chain fructans by bifidobacteria. The majority of *Bifidobacterium* strains studied fermented FOS, as well as low-polymerized inulin, but did not ferment highly polymerized inulin (Biedrzycka & Bielecka, 2004). These results indicate that utilization of inulin by bifidobacteria depends on its degree of polymerization. On the other hand, it has been shown that enzymatic hydrolysis during postharvest storage of Jerusalem artichoke tubers produces depolymerization of inulin into shorter chains (Cabezas et al., 2002; Saengthongpinit & Sajjaanantakul, 2005). Consequently, it is possible to find a correlation between tuber storage time, polymerization degree, and prebiotic capacity of inulin.

The aim of this work was to determine the *in vitro* prebiotic activity of inulin-rich carbohydrates (IRC) extracted from Jerusalem artichoke tubers using *Lactobacillus paracasei* as probiotic microorganism in order to evaluate their utility as prebiotic food ingredient and hence the potential of Jerusalem artichoke as a source of such ingredients for food applications.

2. Materials and methods

2.1. Raw material and chemical reagents

Jerusalem artichoke tubers (*H. tuberosus* L.), Bianca variety, grown and harvested in Córdoba, Argentina, were kindly provided by Instituto Nacional de Tecnología Agropecuaria (INTA), Manfredi Experimental Station. Oligofructose Orafti®P95, inulins Orafti®GR (Inulin GR) and Orafti®HP (Beneo-Orafti, Belgium) were kindly donated by Saporiti SA (Argentina). Fructose, arabinose and sucrose were purchased from Sigma-Aldrich (USA). The reagents and culture media used for prebiotic activity assays were: agar agar, Mann–Rogosa–Sharpe (MRS) broth, Luria–Bertani (LB) broth, Tripticase Soy Agar (TSA), MRS agar, meat extract, yeast extract and proteose peptone (Britania, Argentina), glycerol (Biopack, Argentina) and glucose (Merck, Germany). All the other chemicals used were of reagent grade.

2.2. Tuber handling

Jerusalem artichoke tubers were harvested ten months after planting. Ten days after harvest, the tubers were washed and brushed manually to eliminate soil residues. In order to reduce superficial microorganisms, the tubers were soaked in a 0.038 M sodium hypochlorite solution for 30 min, and then dried with an air stream. This procedure was repeated every 2 months. Clean and dry tubers were packed in plastic woven bags and stored in chamber at 4–5 °C and >98% relative humidity until use. About 2 kg of tubers was taken at 0, 2, 4, and 8 months of storage, respectively. The tubers were cut into slices of 3 mm thickness with a domestic food processor. Slices were frozen at −18 °C and freeze-dried (LA-B3-C, Rificor, Argentina) at −40 °C and 0.014 mm Hg until constant weight (72 h). The lyophilized material was stored in Ziploc® bags under nitrogen atmosphere, and kept in a cool and dry place (15–25 °C, 40% RH) until analysis.

2.3. Soluble solids

Soluble solids were determined refractometrically using a digital refractometer (ATAGO, Tokyo, Japan). The measurements were carried out on tissue juice obtained from tubers after each storage period, using a portable manual press. Jerusalem artichoke tubers apart from free glucose, contain fructose, sucrose and other sugars with different chain lengths (polyfructans) that can contribute in a different way to the refractive index (Frese & Dambroth, 1987).

2.4. Scanning electron microscopy (SEM)

Tubers cold-stored during different periods were freeze-dried and cut into slices (2 mm thickness) and mounted in a double-sided carbon tape placed in the stub. A scanning electron microscope (EVOMA10, Carl Zeiss SMT Ltd., UK) operated at an accelerating potential of 20 kV, was used to visualize the microstructure of the tubers at different magnifications (19–1120×), and representative images were chosen.

2.5. Dry matter

Dry matter (d.m.) of fresh tubers, freeze-dried tubers and ICR powders as well as moisture content of fresh tubers were determined gravimetrically by weighing the samples before and after drying in a vacuum oven (6.4 kPa) at 65 °C until constant weight.

2.6. Tuber composition

Minerals were determined by X-ray fluorescence spectrometry with rhodium anode, helium atmosphere, crystals Fli 200, PX1, PX4, PE and flow detector (Magi'X, PANalytical, The Netherlands). The protein content was determined by the macro-Kjeldahl method, using a digestor and a distillation unit (Official Methods of Analysis of the Association of Official Analytical Chemists, 1990). Fats were extracted with n-hexane (b.p. 68–72 °C) in a Soxhlet apparatus following IUPAC Standard Method 1.122 (1992). Cellulose, hemicellulose and lignin contents were assayed by the technique of acid and neutral detergent (Official Methods of Analysis of the Association of Official Analytical Chemists, 1990, Guiragossian, Van Scoyoc, & Auxtell, 1977). The ash content was evaluated by the technique AOCS Ba 5a-49 (1993).

2.7. Soluble carbohydrate extraction

Freeze-dried slices from tubers were ground in a coffee grinder (Braun, Mexico) to obtain a homogeneous powder. The batch extraction process was performed in a stirred container using distilled water at 85 °C for 2 h, with a solid–solvent ratio of 1:8 (w/v). The solution was separated from the slurry by successive filtrations through muslin cloth and paper filter, at 80 °C. A volume of this aqueous extract was taken for subsequent purification and discoloration, and the rest was freeze-dried and pulverized. The powder obtained was considered to be an IRC sample. Powders obtained from tubers stored during 0, 2, 4, and 8 months were labeled IRC-0, IRC-2, IRC-4, and IRC-8, respectively. Samples were stored at the same conditions as the tuber slices until use.

2.8. Purification and discoloration procedure

A volume of 230 mL of the raw aqueous extract was poured into a beaker, adjusted to pH 11.2 with solid calcium hydroxide, and kept at 65 °C under continuous stirring for 30 min. Then, the temperature was raised to 85 °C, more calcium hydroxide was added until formation of a cloudy precipitate (pH 12), and the solution was stirred for another 45 min. The slurry was filtered and the precipitate discarded. Carbon dioxide was bubbled rapidly into the hot filtrate until the pH reached a value of 10.8. Then the suspension was centrifuged at 3200 rpm for 10 min (Presvac DCA-300, PRESVAC SRL, Argentina). The supernatant was separated by vacuum filtration (S&S filter paper No. 859) at 65 °C. A second carbonation (pH 8.9) and separation steps were carried out under similar conditions. The purified extract so obtained was freeze-dried and pulverized, and the powder obtained was labeled IRCp, where p means purified. It was stored as described above until use.

2.9. Analysis of carbohydrates

The composition analysis of the different IRCp powders was determined by HPLC. Sucrose was determined indirectly by acid hydrolysis

of the IRCp powders. Hydrolysis was performed with HCl 0.01 M (solid–liquid ratio 1:100 w/v) at 90 °C for 6 h, and then the hydrolyzate was neutralized with NaOH 0.01 M. A chromatographic system Alliance e2695 (Waters, USA) was used with an Aminex HPX-87C column (Bio-Rad, USA) 300 mm × 7.8 mm, which was kept at 65 °C. Distilled water was used as mobile phase at a flow rate of 0.3 mL min^{-1}. A refractive index detector was used (Mod. 2414, Waters, USA). The data processing program used was Empower 2 (Shimadzu, Japan). Quantification was performed by comparison of areas through the external standard method, using reference solutions at different concentrations of carbohydrates, ranging from 1 to 10 mg mL^{-1}. The contents of glucose, fructose, sucrose and inulin were determined in IRCp powders and their hydrolyzates. Two solutions were prepared from each IRCp powder, and duplicate HPLC analysis was performed with each solution.

2.10. Carbohydrate extraction yield and inulin content

The carbohydrate extraction yield from tubers (Y) was calculated as the weight of IRCp by the weight of dry tubers, and expressed as %. Inulin content of IRCp (ψ), equivalent to purity of commercial inulin, was calculated as the ratio of the inulin content, as estimated by HPLC, to the weight of IRCp, and expressed as %.

Finally, the inulin content expressed as % per weight of tubers, dry matter (I), was estimated by the expression:

$$I = {}^{Y\psi}\big/{}_{100}.$$ (1)

IRCp extracted from Jerusalem artichoke tubers contains inulin and other carbohydrates (considered impurities), including sucrose (S), free fructose (F_F), and free glucose (G_F). The contents of these sugars were calculated in a similar way as inulin.

The contents of fructose and glucose coming from the hydrolysis of sucrose (F_S and G_S respectively) were calculated by the formula:

$$F_S = G_S = {}^{S}\big/{}_{2}.$$ (2)

The contents of fructose and glucose coming from the hydrolysis of inulin (F_I and G_I respectively) were calculated by the difference (Kocsis et al., 2007):

$$F_I = F_T - (F_F + F_S)$$ (3)

$$G_I = G_T - (G_F + G_S).$$ (4)

2.11. Average degree of polymerization (DPn) of inulin

The average number degree of polymerization (DP_n) of inulin for each ICRp powder was calculated from the following expression, assuming one molecule of glucose per chain of fructan:

$$DP_n = \frac{F_I + G_I}{G_I}$$

$$DP_n = \frac{F_I + G_I}{G_I}.$$ (5)

2.12. Growth and storage conditions of lactic bacteria

L. paracasei BGP1 (CLERICI-SACCO, Cadorago, Italia) culture was used for in vitro prebiotic activity assays of IRC samples. This strain has been reported as a probiotic in previos studies (Huebner et al.,

2007; Verdenelli et al., 2009). Lyophilized microorganism was activated by growing into MRS broth for 24 h under anaerobic conditions at 37 °C. Stock culture was stored at −80 °C in 20% (w/v) glycerol for further use. A percentage of 0.05% of L-cysteine was added to commercial MRS broth in order to improve anaerobic conditions and stimulate the growth of the probiotic.

Frozen stock culture of the microorganism was subcultured overnight in vials with 10 mL of MRS broth with the corresponding sugar and incubated for 12 h at 37 °C under anaerobic conditions. For each assay, a volume of 45 mL of fresh MRS broth containing the different carbon sources to be assayed was inoculated with 100 μL of the corresponding subculture and incubated overnight under the same previous conditions. The assays included a negative control without carbohydrate (basal medium), a positive control with glucose as optimal C source, inulin GR as a reference prebiotic, and the IRC samples from Jerusalem artichoke tubers. In all cases a concentration of 1% (w/v) of carbohydrate was used. This concentration is recommended in the literature as the minimum to ensure the stimulating effect of a carbohydrate on the growth of beneficial bacteria with respect to a basal MRS medium (Li et al., 2008). IRC samples and inulin GR were sterilized by exposition to UV light (254 nm, 40 W) for 15 min before being added to the culture media. The culture was incubated at 37 °C for 24 h under anaerobic conditions. Samples were withdrawn at various times to measure: optical density (OD$_{600}$, Serie SPECTRO-16/18, Shanghai, China), pH (pH meter HI 2211, HANNA, USA), total acidity (% lactic acid), and viable cell counts at 48 h (cfu/mL), determined by the standard plate method under anaerobic conditions at 37 °C. Experiments were performed in triplicate for each assay.

2.13. Relative growth ratio (RGR) of L. paracasei

As a means of assessing the in vitro prebiotic ability of IRC samples, the relative growth ratio of the probiotic L. paracasei was calculated using the expression:

$$RGR = \left(\frac{P_t - P_0}{G_t - G_0}\right)_p$$ (6)

where p subscript indicates probiotic bacteria, P and G represent the growth rates of L. paracasei (log cfu/mL) when the prebiotic or glucose is used as carbon source in the medium, respectively, at time t and 0, as indicated by the corresponding subscript. A RGR value greater than unity indicates that the tested carbohydrate exerts a growth stimulant effect on L. paracasei, in comparison to glucose as C source.

2.14. Growth and storage conditions of enteric mixture

The enteric mixture consisted of three shiga toxin-producing E. coli (STEC) strains: E. coli O157:H7, E. coli O91:H21, E. coli O171:H2 in a 1:1:1 ratio, provided by the Immunochemistry and Biotechnology Laboratory (SAMP-UNCPBA, Tandil, Argentina). This mixture was grown in LB broth at 37 °C for 18 h. Then, tubes containing M9 minimal broth without C source (basal medium, as negative control), 1% (w/v) glucose, 1% (w/v) inulin GR and 1% (w/v) of each IRC samples, respectively, were inoculated with an overnight enteric culture (1%) and incubated at 37 °C for 24 h with stirring. Cultures were plated on TSA at 0 and 24 h of incubation. Each assay was performed in triplicate. The M9 minimal broth contained: 6 g/L Na$_2$HPO$_4$, 3 g/L KH$_2$PO$_4$, 0.5 g/L NaCl, 1 g/L NH$_4$Cl, 1 mL/L MgSO$_4$·7H$_2$0 1 M, and 10 mL/L CaCl$_2$ anhydrous 0.01 M. The medium was adjusted to pH 6.8 ± 0.2 before sterilization. Each test was performed in triplicate.

2.15. Prebiotic activity score

Prebiotic activity indicates the ability of a given substrate to promote the growth of a microorganism relative to other microorganisms and

Table 1
Composition of Jerusalem artichoke tubers.

Components	% $w/w_{d.m.}$		$\mu g/g_{d.m.}$
Ash	6.14 ± 0.57	Mg	27
Lipids	1.16 ± 0.49	Si	65
Proteins	9.78 ± 0.06	P	632
NDF[a]	5.47 ± 0.17	S	216
Lignin	0.52 ± 0.01	Cl	813
Cellulose	2.73 ± 0.14	K	8626
Hemicellulose	2.22 ± 0.02	Ca	279
		Fe	36

[a] Neutral detergent fiber.

relative to growth on non-prebiotic substrates, as glucose. Therefore, carbohydrates have a positive prebiotic activity score if they are fermented as well as glucose by probiotic strains and they are selectively fermented by probiotics but not by other intestinal bacteria. Considering growth rates of *L. paracasei* and *E. coli* attained using glucose, inulin and IRC samples as C source, a prebiotic activity score (PAS) was calculated according to (Huebner et al., 2007) by the expression:

$$PAS = \left(\frac{P_{24}-P_0}{G_{24}-G_0}\right)_p - \left(\frac{P_{24}-P_0}{G_{24}-G_0}\right)_e \qquad (7)$$

where the parenthesis of the right term correspond to RGR as defined by Eq. (6) evaluated al t = 24 h for probiotic (p) and enteric (e) bacteria, respectively.

2.16. Statistical analysis

For each assay, statistical differences were determined by analysis of variance (ANOVA) followed by comparisons between means with Fisher's LSD test, at 5% significance level, using the software InfoStat (Version 2011, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

3. Results and discussion

3.1. Properties of the tubers during storage: composition, moisture and soluble solids

Table 1 shows the composition of Jerusalem artichoke tubers before storage. Considering that inulin represents mostly the soluble fiber content of tubers, these data allowed to estimate a tuber inulin content of 20.6% (w/w), fresh basis (or a 78.1% db). This value is within the average value range found for different varieties of the species (Kaur & Gupta, 2002). Moisture and soluble solid contents of tubers during storage at 4 °C are shown in Table 2. It can be observed that storage time had a significant effect on moisture content, but it had no significant effect on the soluble solid content (°Brix) of the tubers.

As it normally occurs during cold storage of vegetables, moisture content of the tubers decreased with time, in concordance with the results of other authors (Cabezas et al., 2002), while soluble solids were within the range found in a previous work (Saengthongpinit & Sajjaanantakul, 2005), and showed a non-significant increase with

time. Depolymerization of fructans that occurs during cold storage of tubers could explain the increase of soluble fraction of total solids, as in the case of other plant storage organs like onion (Fujishima et al., 2009). Thus, in the early period of storage, the percentage of low molecular soluble carbohydrates was lower than that in the later storage stages. So, tubers of shorter storage periods contained a higher percentage of long chain and less water soluble fructans which could not be detected refractometrically. This result is supported by the fact that F/G ratio remained virtually constant throughout the storage period (Fig. 1).

3.2. Carbohydrate yield and inulin content of IRCp samples

Table 2 presents the values of carbohydrate yield (Y) and inulin content (Ψ) of IRCp samples obtained from tubers cold stored for different periods. Both parameters decreased during storage. The decrease in Y values may be attributed to the consumption of the carbohydrate pool in postharvest biochemical processes that take place during tuber storage. Y value attained for non stored tubers resulted similar to that obtained by Yi et al. (2010) and higher than that reported by Lingyun et al. (2007). These comparisons bring out the influence of extraction method used on the yield of carbohydrates obtained from tubers.

On the other hand, the decrease registered in Ψ values may be a consequence of inulin breakdown. For commercial inulin GR, Ψ value was $94.5 \pm 0.3\%$ (w/w) in accordance with the purity declared by the manufacturer ($\geq 90\%$). This purity level was reached only by the IRCp sample coming from non-stored tubers (IRCp-0). Nevertheless, the other IRCp samples presented purity values $\geq 85\%$, within the range declared for available commercial inulins. In addition, these results are in agreement with other studies on inulin extracted from Jerusalem artichoke tubers (Panchev et al., 2011; Saengthongpinit & Sajjaanantakul, 2005).

3.3. Inulin content of tubers and degree of polymerization of inulin

The levels of carbohydrates at different storage periods of the tubers as derived from HPLC data are shown in Fig. 1. Inulin content decreased with storage time due to the partial enzymatic hydrolysis that degrades it into lower DP fructans, sucrose, glucose and fructose. Experimental inulin content data were fitted with a pseudo first order equation ($R^2 = 0.983$):

$$I = (I_0 - I_\infty) * \exp(-k * t) + I_\infty \qquad (8)$$

where the fitting parameters were the initial inulin content ($I_0 = 32.8\%$ ($w/w_{d.m.}$), the inulin content extrapolated to infinite time ($I_\infty = 23.5\%$ ($w/w_{d.m.}$), and the kinetic constant ($k = 0.324$ s^{-1}). Inulin content decreased approximately 27% during the overall 8 months storage period. Sucrose, fructose, and glucose contents remained virtually constant during storage, with average values of 4.70, 0.20 and 0.09% ($w/w_{d.m.}$), respectively. These results were in agreement with other reports by the different authors for Jerusalem artichoke tubers (Bach et al., 2012; Cabezas et al., 2002; Kocsis et al., 2007; Saengthongpinit & Sajjaanantakul, 2005). The constant levels found for these sugars may be attributed to similar average rates of sugar build up coming from inulin breakdown, and sugar intake used as substrate for respiration and other metabolic activities of the tubers during storage. Cabezas et al.

Table 2
Changes in physicochemical properties of Jerusalem artichoke tubers during storage at 4 °C. Effect on extraction yield (Y) and inulin content (Ψ) of soluble carbohydrates, and average number degree of polymerization (DP_n) of inulin.

Storage time (months)	Moisture (% $w/w_{f.m.}$)	Soluble solids (°Brix)	Y (% $w/w_{d.m.}$)	Ψ (% $w/w_{d.m.}$)	DP_n
0	73.2 ± 1.0 a	23.6 ± 0.0 a	35.0 ± 1.8 a	94.2 ± 3.5 a	12.1 ± 0.9 a
2	72.6 ± 1.0 a	23.5 ± 0.0 a	30.8 ± 1.8 b	90.5 ± 3.8 a	9.8 ± 1.0 b
4	69.0 ± 2.1 b	24.0 ± 0.1 a	29.5 ± 1.3 bc	90.8 ± 5.0 a	9.2 ± 0.3 bc
8	68.3 ± 2.7 b	24.6 ± 0.0 a	28.4 ± 1.3 c	85.0 ± 5.2 b	8.3 ± 0.2 c

Means with a common letter within a column are not significantly different ($p \leq 0.05$).
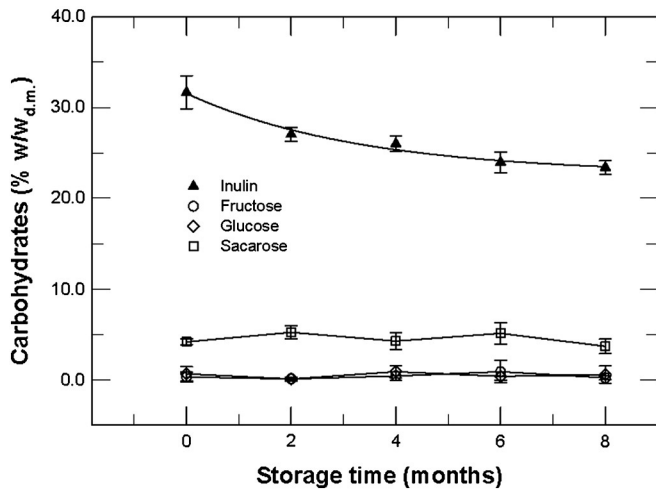
Fig. 1. Inulin and sugar contents (dry basis) in Jerusalem artichoke tubers during storage at 4 °C.

(2002) and Saengthongpinit and Sajjaanantakul (2005) have studied the evolution of fructan and sugar contents during cold storage of Jerusalem artichoke tubers at 4 °C. The first group reported that inulin content decreased to zero after 20 days of storage, glucose content showed a continuous increase, while sucrose and fructose contents showed maximum levels after around 15 days of storage. The differences between these results and ours may be due to the use of tubers with different postharvest periods before to be submitted to cold storage period. More in agreement with our results, Saengthongpinit and Sajjaanantakul (2005) found in Jerusalem artichoke tubers a gradual decrease in inulin fractions of DP > 10 and a gradual increase in sucrose and inulin fractions of DP 3–10 after 4 to 6 weeks of storage.

Calculated values of DP_n are listed in Table 2, which were in agreement with the values reported in previous works (Kocsis et al., 2007; Praznik et al., 2002). It was observed that DP_n decreased during the storage period, indicating degradation of inulin into shorter chains, in accordance with Cabezas et al. (2002), who found a decrease in the fructan fractions of Mw > 1200, and an increase in the fructan fractions of Mw < 1200.

Moreover, the DP_n value reported by the manufacturer for inulin GR is ≥ 10, a value that could be considered similar to those calculated for inulin present in IRCp-0 and IRCp-2 samples.

3.4. Micrograph analysis

Fig. 2 shows the effect of cold storage time on the microstructure of the tubers, obtained by SEM. In the parenchyma of fresh tubers (Fig. 2A) the polygonal cell wall outline was rather rounded and homogeneous, with relatively small cell sizes and thick cell walls. After 2 months of storage (Fig. 2B) cells became more elongated and hexagonal, with thinner cell walls. After 4 months of storage (Fig. 2C) cells resulted more deformed and irregularly sized, which was associated with weakening of the cell walls. After 8 months of storage (Fig. 2D) the cells looked more compacted or shrunk, which was attributed to the loss of vacuolar liquid, in agreement with the loss of moisture of the tubers (Table 2). The dehydration that tubers undergone during cold storage was the main cause of the loss of parenquinal tissue turgor observed in the images, which in turn caused changes in the appearance of the component cells. The overall tissue cellular elongation and its collapse observed during storage were associated with cell wall weakening and loss of water of the tubers.

3.5. Growth of L. paracasei with different carbohydrates sources

Fig. 3A shows the relative growth ratio (RGR) of L. paracasei at different times within 24 h incubation period on MRS medium containing, respectively, IRC samples extracted from Jerusalem artichoke tubers cold stored during 0, 2, 4, and 8 months, and commercial inulin GR as carbon source. At 2 h incubation, the growth of probiotic in all cases was higher than the growth in glucose, except for IRC-0 sample. At t > 2 h of incubation, the growth of the probiotic in most IRC samples was of the same order than the growth in glucose. In particular, IRC-0 sample promoted lower growth ratios compared to glucose during the whole incubation period, which could be attributed to its higher degree of polymerization (DP_n ~ 12) in comparison with the other IRC samples. On the other hand, IRC-4 sample (DP_n ~ 9) allowed obtaining similar or higher growth ratios with respect to glucose during most of the incubation period. These results are in agreement with previous studies
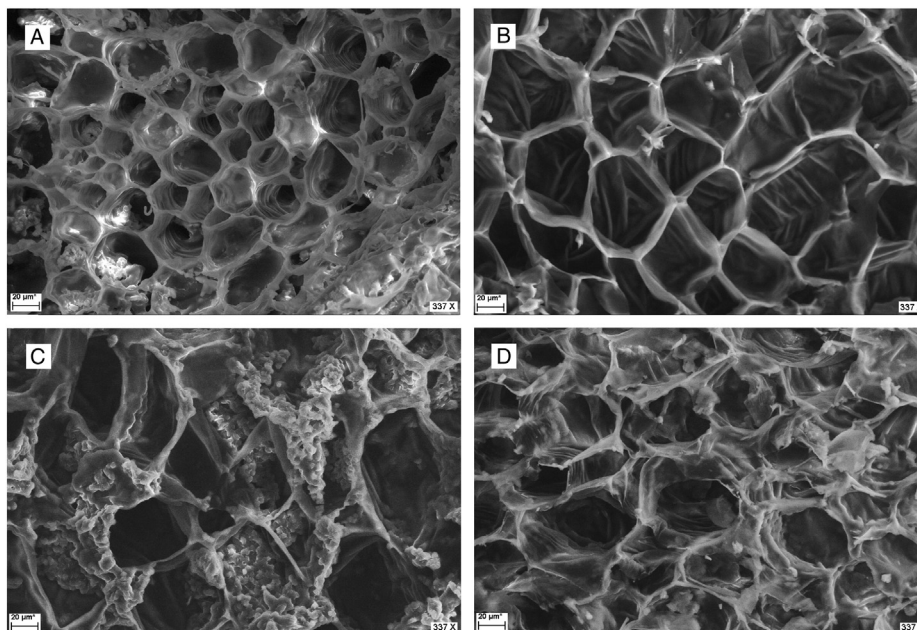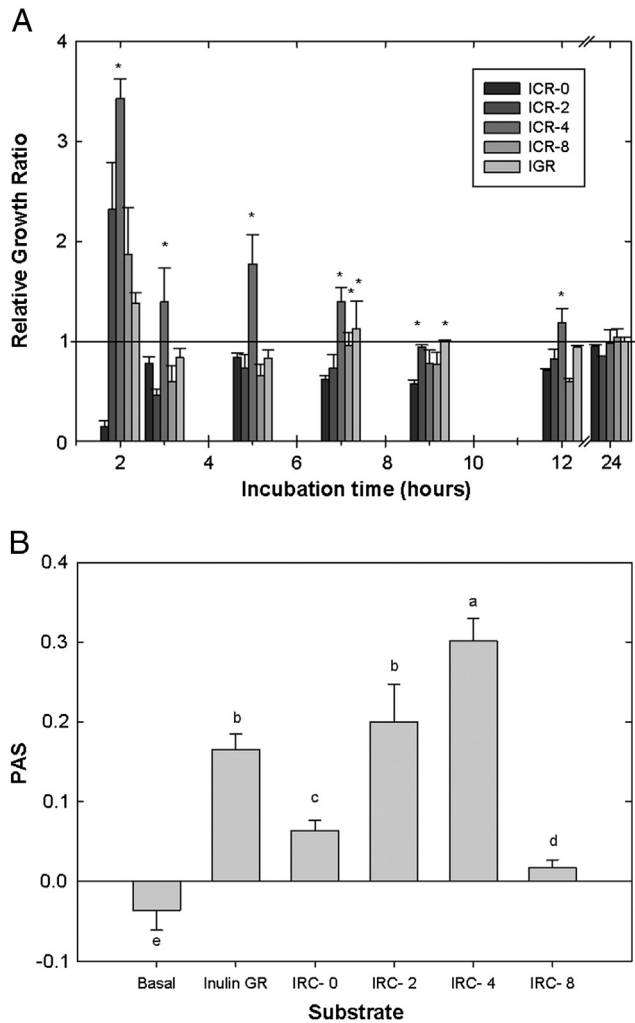

Fig. 2. SEM micrographs (magnification 337×) of the parenchyma of Jerusalem artichoke tubers stored at 4–5 °C and 98% humidity during different times: 0 months (A), 2 months (B), 4 months (C), and 8 months (D).

without C source compared with that obtained on glucose and the fact that the growth of enteric bacteria was less limited by C availability in the medium in comparison to probiotic strain.

The PAS values obtained in this study were indicative of a selective use of IRC samples by *L. paracasei* with respect to pathogenic strains. IRC-4 sample showed the highest PAS value, followed by IRC-2 (which in turn had no significant difference with inulin GR), IRC-0, and IRC-8 samples. Considering that there were no significant differences between RGR values at 24 h obtained with IRC samples as C source (Fig. 3A), differences in the PAS values may be attributed to different growth rates of the enteric bacteria in the respective IRC sample. This result indicated that *L. paracasei* exhibited selectivity for fermentation on individual IRC samples, which followed the order: IRC-4 > IRC-2, inulin GR > IRC-0 > IRC-8.

Total acidity and pH were determined as parameters of the fermentative capacity of the probiotic bacteria when a given carbon source is used in the medium. *In vitro* fermentation of inulin by human colonic bacteria produces lactate and short chain carboxylic acids, mostly acetate (López-Molina et al., 2005). Consequently, the bacterial metabolism of these substrates causes a marked decrease in the culture medium pH. The production of lactic acid from carbohydrates fermentation by *L. paracasei* led to a decrease of pH of the growing media. Fig. 4A shows the pH variation (ΔpH) as a function of incubation time for the different substrates studied. It can be observed that the pH of the

Fig. 3. A) Relative growth ratio of *L. paracasei* (Eq. (6)), as a function of incubation time in anaerobiosis at 37 °C, on different inulin-rich carbohydrate samples (IRC-0, IRC-2, IRC-4 and IRC-8) and commercial inulin (IGR). Significant differences (p < 0.05) between samples are indicated with an asterisk. The horizontal line represents a growth of *L. paracasei* on inulin samples equivalent to that attained with glucose. B) Prebiotic activity score (PAS) calculated with Eq. (7) for the different substrates. Samples with a common letter are not significantly different (p = 0.05).

(Biedrzycka & Bielecka, 2004), which showed that highly polymerized inulins ($DP_n > 10$) are fermented more slowly by *Bifidobacterium* strains than fructans with shorter chains ($6 < DP_n < 10$). Finally, despite its similar degree of polymerization ($DP_n \sim 8–9$), IRC-8 sample induced lower growth rates than IRC-4 sample, which may be attributed to the lower inulin content of the former (Table 2).
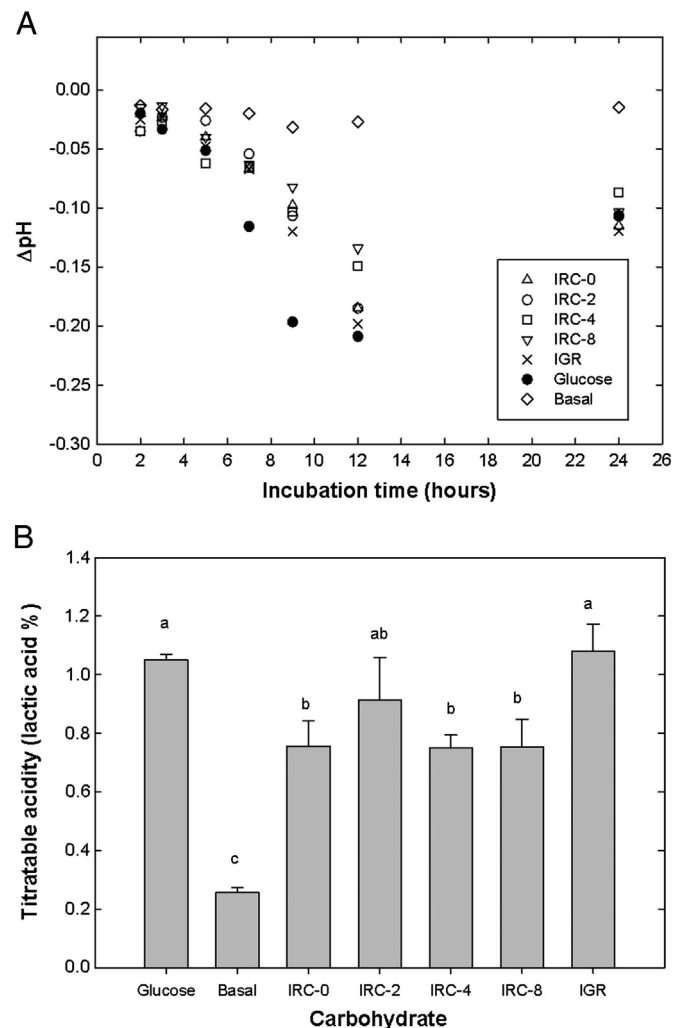
3.6. Assessment of prebiotic properties

Fig. 3B shows the prebiotic activity score (PAS) values obtained for different IRC samples, commercial inulin GR, and the corresponding to a control assay without C source (basal media). The PAS value arises from the difference between the relative growth of the probiotic at 24 h using prebiotic and glucose as C source, respectively, and the relative growth of the pathogen at 24 h using prebiotic and glucose as source of C, respectively. The higher the score, the higher the relative growth of the probiotic and/or the lower the relative growth of the pathogen, which indicates a higher and more selective use of prebiotic in relation to glucose by the probiotic microorganism and/or a limited use of prebiotic in relation to glucose by the pathogenic microorganism. A negative PAS value was obtained for the basal medium. Factors that contributed to this result were a less favored growth of probiotic on the medium

Fig. 4. A) pH variation of the MRS substrate with 1% of different carbohydrates, as a function of the incubation time in anaerobiosis at 37 °C. B) Titratable acidity of the different substrates at 24 h of incubation in anaerobiosis at 37 °C. Samples with a common letter are not significantly different (p = 0.05).

MRS basal media remained almost constant during 24 h. With all other substrates, pH of the media decreased during the first 12 h due to carbon source fermentation, and then increased at 24 h, which may be consequence of biochemical processes that took place during advanced growing and death stages of the bacteria. It can also be observed that there were no significant differences between the ΔpH of the media attained with different IRC samples, including commercial inulin GR. The ΔpH obtained when glucose (non prebiotic) was used as substrate was significantly low at 7 and 9 h of incubation, with the lowest value at 12 h, in concordance with the time of the highest growth rate of the probiotic.

Fig. 4B shows the total acidity of *L. paracasei* growing media attained with different carbon sources at 24 h of incubation, expressed as lactic acid % (w/v). No significant differences were found between the values attained with different IRC samples. However, acidity obtained with commercial inulin GR and glucose were both significantly higher than the values corresponding to IRC samples, except for IRC-2. In spite of these differences, results indicated that both commercial inulin and IRC samples were fermented by *L. paracasei*.

4. Conclusions

This work describes a method for the extraction of inulin-rich carbohydrates from Jerusalem artichoke tubers, that includes several steps carried out exclusively in aqueous medium, without the addition of any organic solvents. Inulin-rich carbohydrates extracted from tubers stored during different times at 4–5 °C and 98% relative humidity along an overall period of 8 months, were fermented by the probiotic bacteria *L. paracasei*. The data obtained allowed us to conclude that inulin-rich carbohydrates samples were fermented by *L. paracasei* as efficiently as commercial inulin obtained from chicory roots. In particular, inulin-rich carbohydrates extracted from tubers stored during 4 months exhibited the highest *in vitro* prebiotic activity score, even higher than the value obtained using the commercial inulin. These results indicated that this sample met the conditions, including purity (~90% w/w) and degree of polymerization (~9) required to be considered as a potential prebiotic food ingredient.

This study represents a contribution for selecting plant species as feasible sources of prebiotic ingredients. Moreover, the results obtained put in evidence the influence of storage conditions of raw material on the functional properties of derived ingredients. Additional research is required to establish the *in vivo* prebiotic capacity of inulin-rich carbohydrates aiming its inclusion in functional food development.

Abbreviations

DP	degree of polymerization
DP_n	average number DP
IRC	inulin-rich carbohydrates
IRCp	purified IRC
PAS	prebiotic activity score
RGR	relative growth ratio

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