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Molecular properties and prebiotic effect of inulin obtained from artichoke (*Cynara scolymus* L.)

Dorotea López-Molina [a], María Dolores Navarro-Martínez [a], Francisco Rojas Melgarejo [b], Alexander N.P. Hiner [c], Soledad Chazarra [c], José Neptuno Rodríguez-López [a,*]

[a] *Grupo de Investigación de Enzimología, Departamento de Bioquímica y Biología Molecular A, Facultad de Biología, Universidad de Murcia, E-30100 Espinardo, Murcia, Spain*
[b] *Grupo de Química de Carbohidratos y Biotecnología de Alimentos (QCBA), Departamento de Química Orgánica, Facultad de Química, Universidad de Murcia, E-30100 Espinardo, Murcia, Spain*
[c] *Artbiochem S.L., Jacinto Benavente 4, E-30600 Archena, Murcia, Spain*

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Abstract

A high molecular weight inulin has been prepared from artichoke (*Cynara scolymus* L.) agroindustrial wastes using environmentally benign aqueous extraction procedures. Physico-chemical analysis of the properties of artichoke inulin was carried out. Its average degree of polymerization was 46, which is higher than for Jerusalem artichoke, chicory, and dahlia inulins. GC–MS confirmed that the main constituent monosaccharide in artichoke inulin was fructose and its degradation by inulinase indicated that it contained the expected β-2,1-fructan bonds. The FT-IR spectrum was identical to that of chicory inulin. These data indicate that artichoke inulin will be suitable for use in a wide range of food applications. The health-promoting prebiotic effects of artichoke inulin were demonstrated in an extensive microbiological study showing a long lasting bifidogenic effect on *Bifidobacterium bifidum* ATCC 29521 cultures and also in mixed cultures of colonic bacteria.

Keywords: Inulin; Artichoke; Bifidobacteria; Prebiotic effect; Inulinase

1. Introduction

Inulin is a plant-derived carbohydrate with the benefits of soluble dietary fiber. It is not digested or absorbed in the small intestine, but is fermented in the colon by beneficial bacteria. Functioning as a prebiotic, inulin has been associated with enhancing the gastrointestinal system and immune system. In addition, it has been shown to increase the absorption of calcium and magnesium, influence the formation of blood glucose, and reduce the levels of cholesterol and serum lipids (Coudray et al., 1997; Niness, 1999). Therefore, inulin obtained from several Compositae (Jerusalem artichoke, artichokes, chicory, dahlias, and dandelions) is a subject of interest in many food research programs (Mitchell and Mitchell, 1995; Smits and Hermans, 1998; Silver and Brinks, 2000; Heyer et al., 1998; van Loo and Hermans, 2000). Inulin is not simply one molecule; it is a polydisperse β-2,1 fructan (Phelps, 1965). The fructose units (F) in this mixture of linear fructose polymers and oligomers are each linked by β-2,1 bonds. A glucose molecule (G) typically resides at the end of each fructose chain and is linked by an α-1,2 bond, as in sucrose. The unique aspect of the structure of inulin is its β-2,1 bonds.

* Corresponding author. Tel: +34 968 398284; fax: +34 968 364147.
E-mail address: neptuno@um.es (J.N. Rodríguez-López).

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These linkages prevent inulin from being digested like a typical carbohydrate and are responsible for its reduced caloric value and dietary fibre effects.

Inulins obtained from different plants differ in their degree of polymerization (DP). As inulin is a polydisperse mixture of oligomers with varying DPs, inulin samples are characterized by their average degree of polymerization, DP_n. The differences in DP_n between different inulins account for their distinctly different functional attributes. Long chain length inulins are less soluble, and they have the ability to form inulin microcrystals when sheared in water or milk. These crystals are not discretely perceptible in the mouth, but they interact to form a smooth creamy texture and provide a fat-like mouth sensation. Inulin has been used successfully to replace fat in table spreads, baked goods, fillings, dairy products, frozen desserts and dressings.

Globe artichokes (*Cynara scolymus* L.) are perennial, frost sensitive, thistle-like plants with edible flower buds, which sprout from the terminal portion of the main stem and on lateral stems. Each unopened flower bud resembles a deep green pine cone, 7–10 cm in diameter, round, but slightly elongated. Several pointed, leathery green bracts fold around a purple-blue flower. The base of each bract is the fleshy edible portion, along with the fleshy centre of the artichoke on which the flower and bracts are borne. Commercially available inulins are obtained mainly from chicory, Jerusalem artichoke, and dahlia. They are distributed with several trade names such as Raftiline or Fibruline. Although artichokes possess high inulin content [3% of fresh weight (van Loo et al., 1995)] its properties and possible applications are less well known than those from other sources probably because the flowers of the plant are usually eaten as a vegetable. Our research group in collaboration with the Spanish company Artbiochem, S.L., has designed a protocol for the isolation and purification of inulin from artichoke waste materials (bracts) from the canning industry. The artichoke canning industry, an active sector in our local area, generates large amounts of industrial waste, consisting mainly of the stems and external parts of the flowers (bracts) of the artichoke plant, which are not suitable for human consumption. For example, 70% of the weight of the artichoke flower corresponds to waste. Said wastes are generally used in the production of animal feed, particularly silage. Therefore, isolation of a by-product with high added value, such as inulin, from these industrial wastes is of commercial interest. The process we have developed is carried out exclusively in aqueous medium, without the addition of any organic solvents, which is environmentally friendly in itself and could be of importance for the use of this inulin in foods. Therefore, in this study we have characterized artichoke inulin and explored its possible application as a prebiotic agent. Moreover, further investigations are in progress to determine the possible inclusion of this inulin in other applications including diagnostic reagents, preparation of specific drug carriers and its use as an anticarcinogenic agent (Hughes and Rowland, 2001; Chourasia and Jain, 2003).

2. Results and discussion

2.1. Extraction of artichoke inulin

The initial inulin extract was obtained from milled artichoke bracts in aqueous solution. The liquid extract was filtered and concentrated by ultrafiltration using a 10000 NMWCO membrane to obtain a high molecular weight fraction of artichoke inulin. At this stage most of the acid components, pigments, and dyes were removed from the crude precipitate by ion-exchange chromatography, yielding a colourless material. The concentrated extract was then submitted to a precipitation process at low temperature and subsequent phase splitting by centrifugation. Variables involving inulin precipitation yield (w/w) were identified and studied: time for precipitation, precipitation temperature (from −24 to 16 °C), centrifugation velocity (from 6000 to 10,000*g*) and centrifugation time (from 15 to 45 min). The results showed a tendency to increased inulin precipitation mass yield when precipitation time increased and temperature decreased, independently of centrifugation velocity and time. The time-course of inulin precipitation showed maximal yield after 12 h at −24 °C. The final lyophilized white powder was subjected to several characterization techniques.

2.2. Physico-chemical properties of artichoke inulin

The physico-chemical characteristics of artichoke inulin are compared with samples of standard and high performance chicory inulin in Table 1. Artichoke inulin is moderately soluble in water (maximum 5% at room temperature), it has a bland neutral taste, without any off-flavour or aftertaste, and is not sweet. Therefore, it combines easily with other ingredients without modifying delicate flavours. For reasons of growing interest in the food and pet food industries, the short chain inulins have to be separated from their long chain analogues, because their properties (digestibility, prebiotic activity and health promoting potential, caloric value, sweetening power, water binding capacity, etc.) differ substantially (van Loo and Hermans, 2000; van Leeuwen et al., 1997; De Gennaro et al., 2000). The method applied here for artichoke inulin preparation produced high molecular weigh fractions of the polymer, and made further fractionation procedures (Moerman et al., 2004) by precipitation from water/solvent

Table 1
Physico-chemical characteristics of chicory and artichoke inulin

	Standard chicory inulin[a]	High performance chicory inulin[a]	Artichoke inulin
Average degree of polymerization	12	25	46
Dry matter (%)	95	95	95
Inulin/oligofructose content (% on d.m.)	92	99.5	99.5
pH (10% w/w)	5–7	5–7	5–7
Sulphated ash (% on d.m.)	<0.2	<0.2	<0.2
Heavy metals (ppm on d.m.)	<0.2	<0.2	<0.2
Appearance	White powder	White powder	White powder
Taste	Neutral	Neutral	Neutral
Sweetness (v. sucrose = 100%)	10%	None	None
Solubility in water at 25 °C (g/l)	120	25	5

d.m.: Dry matter.
[a] Data obtained from Franck (2002).

mixtures unnecessary. The process is ideal for food applications. Artichoke inulin showed a high DP_n value ($DP_n = 46$) when compared with inulins from different sources ($DP_n = 26$, 24, and 43 for Jerusalem artichoke, chicory, and dahlia inulin, respectively). The high average degrees of polymerization of artichoke inulin make its properties closer to those of high performance chicory inulin and it could be used for similar applications in the food industry. For instance, when used for replace fat inulin, mixed with water or an aqueous solution, forms a particle gel network resulting in a creamy structure with a spreadable consistency, which can easily be incorporated into foods to replace up to 100% of the fat (Franck, 2002). Artichoke inulin could also be used in combination with gelling agents such as gelatin, alginate, k- and i-carrageenans, gellan gum and maltodextrins. It also improves the stability of foams and emulsions, such as aerated desserts, ice creams, table spreads and sauces. This inulin could, therefore, replace other stabilisers in different food products (Franck, 2002).

The hydrolysate of this material showed a major content of fructose and a smaller one of glucose as determined by GC–MS experiments. A comparison of gas chromatograms for different inulins is shown in Fig. 1. Three major peaks with retention times at 10.1 ± 0.04, 10.3 ± 0.01 and 11.65 ± 0.03 min were characteristic for the hydrolysates of all the inulins studied. Mass spectra of these peaks correspond to fructose with authentic samples of this monosaccharide used as standard. The FT-IR spectrum for artichoke inulin was essentially identical to chicory inulin (Fig. 2) showing OH stretch (3353 cm^{-1}) and carbonyl bands (1745 cm^{-1}) characteristic of inulin (Wu and Lee, 2000).

2.3. Degradation by inulinase

Artichoke inulin was incubated with inulinase from *Aspergillus niger* which is a mixture of *exo*- and *endo*-inulinase. The enzyme mixture hydrolyzes linear β-2,1-linked fructose polymers of inulin initiated by a glucose unit. Most inulinases are β-fructosidases and split-off fructose moieties from the non-reducing end of the inulin molecule or from certain sugars displaying a fructose unit at the terminal β-2,1-position. These enzymes can be denominated as 2,1-β-D fructan-fructano hydrolases. Inulinases with β-fructosidase activity are encountered in plants and in microorganisms, including fungi, yeast, and bacteria. Several methods have been proposed for oligosaccharide analysis (Dysseler and Hoffem, 1995). In order to study the enzymatic degradability of artichoke inulin we performed thin layer chromatography (TLC) (Damian et al., 1999) and the results are shown in Fig. 3. After 12 h at 37 °C artichoke inulin was completely degraded to fructose units by inulinase. Similar results were obtained with chicory inulin (Fig. 3). The results indicated that artichoke inulin could be used as a source of fructose and oligofructose by addition of inulinase by controlling the time of degradation.

2.4. Prebiotic effect of artichoke inulin

Perhaps the best-known nutritional effect of inulin is its stimulation of bifidobacterial growth in the intestine. The colon is known to be a complex ecosystem with >400 different types of bacteria. Some strains have pathogenic effects such as the production of toxins and carcinogens, whereas others are considered to provide a health-promoting function: among those in this second group are lactobacilli and bifidobacteria. Feeding beneficial bacteria, such as bifidobacteria, with inulin allows them to "outcompete" potentially detrimental organisms and thereby contribute to the health of the host. Health benefits ascribed to bifidobacteria include the following: inhibiting the growth of harmful bacteria, stimulating components of the immune system, aiding the absorption of certain ions and the synthesis of B vitamins. The bifidogenic effect of inulin

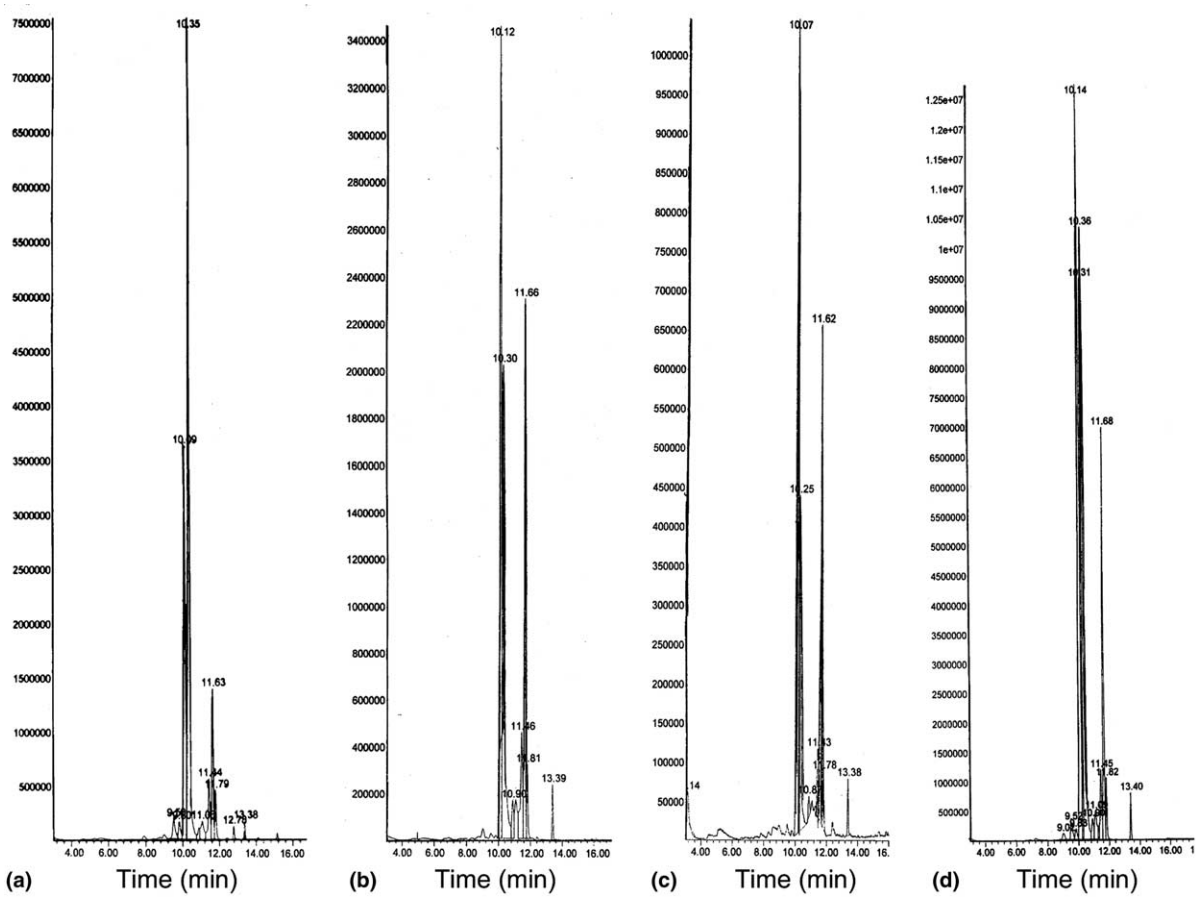

Fig. 1. GC–MS results for artichoke inulin (a) compared with chicory (b), dahlia, (c) and Jerusalem artichoke (d) inulins.

has been well proven (Djouzi and Andrieux, 1997; Gibson and Roberfroid, 1995; Roberfroid et al., 1998). Dramatic positive shifts in the composition of microflora have been shown through in vivo human studies at doses between 5 and 20 g/day, generally over a 15-day period (Wang and Gibson, 1993; Gibson et al., 1995).

In order to demonstrate the bifidogenic effect of artichoke inulin we have studied the effect of its addition in cultures of fresh faecal slurry from healthy infant donors containing health-promoting bacteria and pathogenic bacteria (Fig. 4). In vitro fermentation of inulin by human colonic bacteria, mainly bifidobacteria, produces lactate and short chain carboxylic acids, mostly acetate. Consequently, the bacterial metabolism of these substrates causes a marked decrease in the culture medium pH (Table 2). In vitro experiments on the comparative fermentation of chicory inulin and artichoke inulin in anaerobic bath cultures inoculated with 200 g/l mixed infant faecal bacteria and 20 g/l of the different carbohydrates have been conducted. The data reported in Fig. 4 show that both inulins were fermented by the microbial flora. Moreover, the relative rate of fermentation was similar for both substrates. The utilization of artichoke inulin by the same microorganisms, *Bifidobacterium* spp., *Bacteroides fragilis*, *Lactobacillus* spp., *Escherichia coli* and total anaerobes was comparable to chicory inulin and to the control with no added inulin. Bacterial growth was slower but longer-lasting in the presence of both chicory and artichoke inulin compared to the control with glucose.

In order to prove that artichoke inulin is effectively digested by bifidobacteria from the human colon we have studied the effect of its addition to cultures containing *Bifidobacterium bifidum* ATCC 29521, with a MacFarland turbidity sufficient to give a concentration of 10^6 colony-forming units/ml (CFU/ml) at the beginning of the experiment, (Fig. 5(a)). Similar cultures with chicory inulin were used for comparison and with glucose as the control. The results showed that the growth behaviour of *B. bifidum* was similar with either inulin. The inulin-free control showed decreased growth with time, so the addition of any kind of inulin has a long-lasting bifidogenic effect. A pH decrease correlates with the population growth (Fig. 5(b)), indicating the production of acetic and lactic acids (Scardovi, 1986). Moreover, the lower pH has an additional and

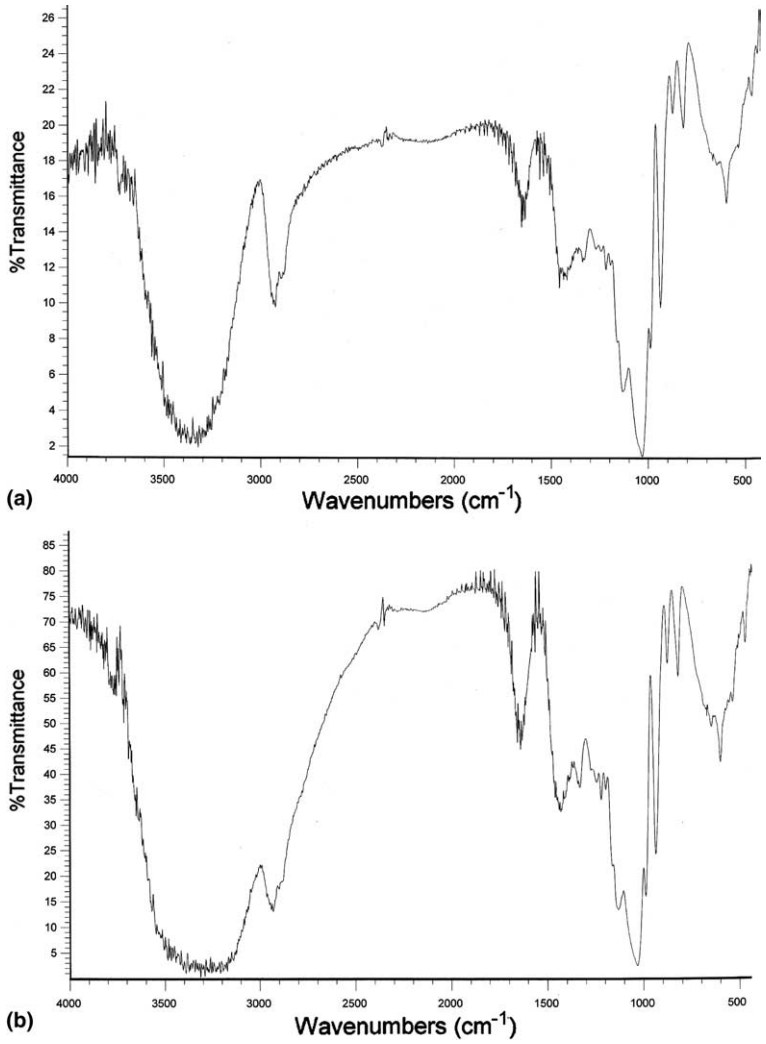

Fig. 2. FT-IR spectrum for inulin from artichoke (a) and chicory (b).

potentially more important effect because the production of these acids reduces intestinal pH and restricts or prohibits the growth of many potential pathogens and putrefactive bacteria.

3. Conclusion

In conclusion, this work describes a method for the preparation of a high molecular weigh fraction of inulin from artichoke waste materials. The process includes several steps carried out exclusively in aqueous medium, without the addition of any organic solvents, which is environmentally friendly in itself and could be of importance for the use of this inulin in food. Artichoke inulin presents similar physico-chemical properties to high performance chicory inulin but an even higher degree of polymerization, this make artichoke inulin appropriate for applications in the food industry. Fat and carbohydrate replacement with artichoke inulin could offer the advantage of not compromising taste and texture, while delivering nutritionally enhanced products.

4. Experimental

4.1. Plant material and chemicals

Artichoke bracts, obtained as agroindustrial waste, were frozen in liquid nitrogen to enhance the crushing and grinding (carried out using an industrial food mixer) of this fiber-rich material and stored at −20 °C until use. Standard grade inulins from Jerusalem artichoke, dahlia, and chicory were obtained from Sigma Chemical Co. (Madrid, Spain) and used as provided. All chemicals

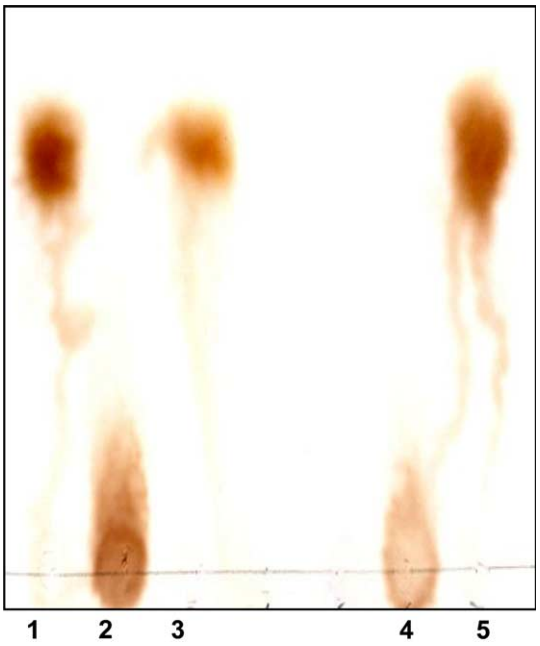

Fig. 3. TLC analysis of fructose (1), chicory inulin (2), chicory inulin plus inulinase (3), artichoke inulin (4), and artichoke inulin plus inulinase (5).

were of analytical grade and purchased from Sigma–Fluka–Aldrich.

4.2. Extraction of artichoke inulin

A process for the production of a high molecular weigh fraction of inulin from artichoke bracts was developed in co-operation with Artbiochem, S.L. The process involved several physical steps (all in aqueous media) including filtration, ultrafiltration (10,000 kDa nominal molecular weight cut-off, NMWCO), ionic chromatography all steps at 4 °C, low temperature precipitation and lyophilization.

Table 2
pH evolution in ferments of faecal slurry with different carbohydrates

Time (h)	pH		
	Control	Chicory inulin	Artichoke inulin
0	7.43	7.55	7.47
6	4.60	4.52	5.46
24	4.54	4.45	4.68
48	4.24	4.25	4.53

4.3. Determination of the DP_n

The procedure to determine the DP_n is based on the principles of end group analysis. DP_n is the value that corresponds to the total number of saccharide units (G and F units) in a given inulin sample divided by the total number of inulin molecules, without taking into account the monosaccharides glucose (G), fructose (F), and the disaccharide sucrose (GF), which are possibly present in the sample. To determine the average DP of the standard grade and fractionated inulin samples, their glucose and fructose content were quantified after hydrolysis with perchloroacetic acid (PCA). For that purpose, an enzyme test kit from Sigma was used (Degenaar et al., 1987). Analysis of D-glucose always proceeded according to the same principle: hexokinase, the first enzyme in the kit, catalyzes the phosphorylation of D-glucose to glucose-6-phosphate, which with the aid of glucose-6-phosphate dehydrogenase and nicotinamide

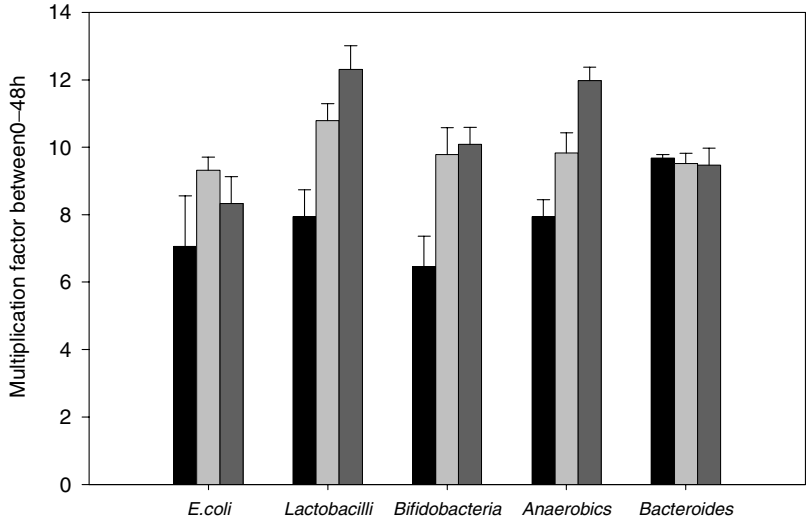

Fig. 4. Changes in the values of colony forming units per milliliter expressed as multiplication factor of infant faecal bacteria after incubation for 48 hours in the presence of 2% chicory inulin (light grey), 2% artichoke inulin (dark grey), and control (black).

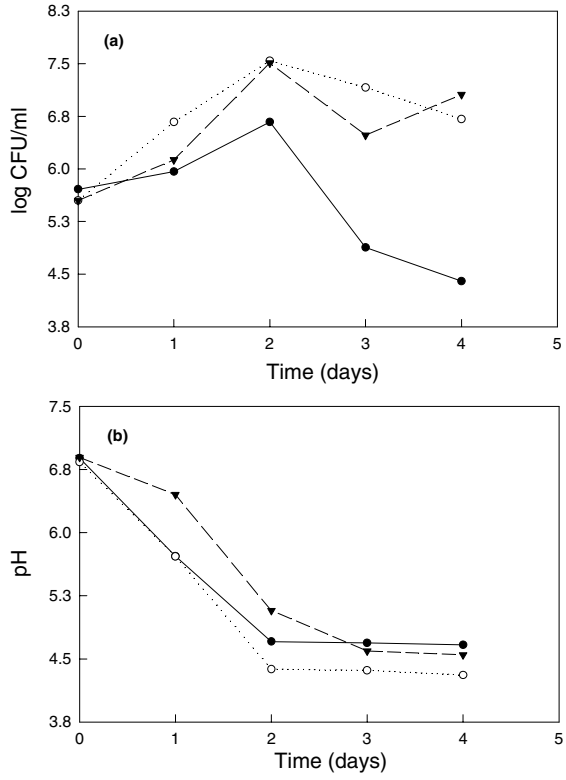

Fig. 5. (a) Population growth and (b) pH decrease of in three cultures of *Bifidobacterium bifidum* with glucose (●), chicory inulin (○), and artichoke inulin (▼).

adenine dinucleotide phosphate (NADP) is further specifically oxidized to D-gluconate-6-phosphate. According to the stoichiometry of the last reaction, the photospectrometrically quantified amount of reduced nicotinamide adenine dinucleotide phosphate (NADPH) is representative for the amount of D-glucose. D-Fructose was determined subsequently to the determination of D-glucose. With the same hexokinase, D-fructose undergoes phosphorylation to fructose-6- phosphate, which is converted to glucose-6-phosphate by phosphoglucose isomerase. Oxidation to D-gluconate-6-phosphate as described above generates a supplementary amount of NADPH that is stoichiometric with the amount of D-fructose. By calculating the fructose-to-glucose ratio (number of fructose units per number of glucose units), the average DP was obtained:

$$\mathrm{DP} = \text{number of F units per G units} + 1 \text{ G unit}$$

$$\mathrm{DP} = C_{\mathrm{fructose}}/C_{\mathrm{glucose}} + 1$$

4.4. Gas chromatography–mass spectrophotometry (GC–MS) analyses

Mass spectra (70 eV) were obtained with a Hewlett–Packard gas chromatograph model 5890 coupled to a mass spectrometer model 5972. GC was performed with a 30 m length and 0.250 mm diameter HP-5MS column. Helium was used as the carrier gas, at a flow of 1 ml/min. The temperature of the oven was programmed from 250 to 280 °C at 10 °C/min and then isothermal. Inulin samples at a concentration of 1 mg/ml were prepared in anhydrous methyl alcohol and completely hydrolyzed at 80 °C for 1 h after the addition of 0.02 ml of this sample to 0.48 ml of methanoic acid. TMS volatile derivatives of inulin hydrolysate were prepared in 0.1 ml of pyridine as solvent by adding 0.1 ml hexamethyldisilazane and 0.05 ml of trimethylchlorosilane (Sweely et al., 1963). Identification of fructose and glucose was confirmed by comparing their GC–MS spectra with those of authentic samples.

4.5. Infrared spectroscopy

Fourier-transform infrared (FT-IR) spectra were obtained on a NICOLET Magna 750 spectrometer with DTGS detector and OMNIC 3.2 software using 128 scans at a resolution of 4 cm^{-1}. The samples (2 mg) were pressed into pellets of KBr (200 mg).

4.6. Degradation of inulins by inulinase

Chicory or artichoke inulins (0.1 g) were mixed with 10 ml of citrate buffer (50 mM) of pH 4.8 in the absence or presence of inulinase (5 units) from *A. niger* (Sigma). The samples were incubated at 37 °C for 12 h, after which they were centrifuged and the supernatant was used for further TLC analysis. The presence of sugars in the solution medium was analyzed by TLC on silica gel plates (Merck 60 F254) with a solvent system consisting of 1-butanol:acetic acid:water (3:3:2; v/v). Development proceeded for 1 h. After drying the plates, sugar spots were detected by spraying a reagent containing Orcinol® (10 mg/ml) in 5% (v/v) H_2SO_4 followed by heating for 15 min at 100 °C.

4.7. Bath culture fermentations for faecal slurries

The basic culture medium was the same as that used by Olano-Martín et al. (2000), containing (g/l): peptone water, 2; yeast extract, 2; NaCl, 0.1; K_2HPO_4, 0.04; KH_2PO_4, 0.04; $MgSO_4 \cdot 7H_2O$, 0.01; $CaCl_2 \cdot 6H_2O$, 0.01; $NaHCO_3$, 2; hemin (dissolved in a few drops of 1 M NaOH), 0.05; cysteine · HCl, 0.5; bile salts, 0.5; Tween 80, 2; 10 μl vitamin K1. The medium was adjusted to pH 7.5 using 1 M HCl. The method used was modified from Olano-Martín et al. (2000). To sterile bottles containing 4 ml culture medium, glucose, chicory inulin or artichoke inulin were added prior to inoculation to give a final concentration of 2%. The bottles were inoculated with 1 ml of infant faecal slurry. Faecal slurry was prepared using fresh faeces from healthy baby donors (who had not taken antibiotics) pre-reduced in

tryptone water and homogenized for 2 min in a stomacher. After mixing and capping, the bottles were introduced into four different anaerobic jars (Oxoid) with AnaeroGenTM 2.5 l sachets (Oxoid). The anaerobic jars were incubated at 37 °C. Samples were removed at 0, 6, 24 and 48 h for enumeration of bacteria. For bacterial enumeration, samples were serially diluted to 10^{-9} in an anaerobic cabinet with pre-reduced tryptone water and inoculated onto a range of agars designed to be selective for predominant colonic bacteria. Bacteria were counted on: Plate Count agar (Oxoid; total aerobes); Schaedler anaerobe agar (Oxoid; total anaerobes); MRS agar (Oxoid; *Lactobacillus* spp.), BSM agar (Fluka; *Bifibobacterium* spp.), Sorbitol MacConkey agar (Oxoid; *E. coli*); Brucella agar (Oxoid; *B. fragilis*). Various antibiotics were added to the agars in order to improve selectivity. The dishes were introduced into anaerobic jars at 37 °C and CFU were counted after 48 h of incubation.

4.8. Bath culture fermentation for prebiotic effect

B. bifidum ATCC 29521 was conserved frozen at −80 °C in glycerol–meat medium and inoculated onto BSM Agar 96 h and 48 h prior to use. The culture medium used was BSM-Broth (Fluka) containing peptone and meat extract as sources of carbon, nitrogen, vitamins and minerals. B-Complex vitamins were supplied by yeast extract to stimulate bacterial growth. The source of carbohydrate was dextrose and sodium chloride maintains the osmotic balance; components including antibiotics inhibit the growth of moulds, enterococci and Gram-negative bacteria. The medium had a final pH of 6.8 ± 0.2. Glucose or inulins were added individually before inoculation to give a final concentration of 2%. Sterile bottles containing 25 ml of BSM Broth medium were inoculated with 250 µl of a solution of *B. bifidum* ATCC 29521 (MacFarland 0.5) mixed and capped and introduced into 3 anaerobic jars as above. The anaerobic jars were incubated at 37 °C. Samples were removed at 0, 24, 48, 72 and 96 h to enumerate the bacteria. The inulins used were from chicory root (Sigma) and artichoke (Artbiochem, S.L.); a BSM broth medium without inulin but with glucose was used as control. For bacterial enumeration, samples were serially diluted to 10^{-7} as above and inoculated onto BSM agar with BSM supplement in order to improve selectivity. The dishes were incubated and CFU counted as above.

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