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Products of thermolysis of potato starch treated with hydrochloric and citric acids as potential prebiotics

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RESEARCH ARTICLE

Abstract

New starch preparations were produced by thermolysis of potato starch in the presence of inorganic (hydrochloric) and organic (citric) acids at 150 °C for 3 h and 180 °C for 1 h. The resulting dextrins were physicochemically and structurally characterised and analysed for their resistance to enzymatic digestion *in vitro*. Moreover, dextrins were tested as products with potential prebiotic properties, selectively stimulated growth and activity of beneficial bacteria, band not contributed to growth of undesirable intestinal microflora. It was found that depolymerisation of the starch was not accompanied by significant chemical modification. Citric acid acted as activator or catalyst for repolymerisation and polycondensation of glucose polymers with low molecular weight saccharides to form non-digestible products. The studies indicated possibility of preparation of well-soluble dextrins, with low water binding capacity, of low pH and very low viscosity, containing about 80% of molecules with weight average molecular weight of 3,500 g/mol (average degree of polymerisation ≅ 22), with reduced caloric value in comparison to native starch, as a result of their resistance to enzymatic hydrolysis. Microbial studies revealed that beneficial *Lactobacillus* and *Bifidobacterium*, in the presence of dextrins prepared with citric acid, were able to dominate the environment in the common culture with other bacteria isolated from human faeces. The results confirmed the possibility of applying dextrins, prepared under specific conditions, as soluble dietary fibre and after fulfilment of additional requirements – also prebiotics.

Keywords: beneficial bacteria, dietary fibre, dextrins, enzyme resistance

1. Introduction

As a carbohydrate occurring in food products, especially cereal- and potato-based ones, starch provides the organism with necessary energy. As a result of the activity of amylolytic enzymes of the gastrointestinal tract starch undergoes hydrolysis. Hence it is regarded a compound rapidly and completely digested and absorbed in the small intestine in the form of glucose being a product of hydrolysis (Gray, 2003; Leszczynski, 2004; Oku and Nakamura, 2002). It has been shown, however, that it only refers to starch subjected to thermal treatment in an appropriate amount of water (i.e. in a gelatinised form) and consumed immediately after being prepared (Leszczynski, 2004). Therefore starch has been divided into: (1) rapidly digestible starch, degraded to glucose within 20 min upon enzymatic activity; and (2) slowly digestible starch, degraded to glucose within the successive 100 min. Some part of consumed starch has been observed to be incompletely digested and in the intact form or as products of its partial hydrolysis to escape the small intestine and enter the large bowel. This part of starch has been termed 'resistant starch' (RS). By definition, resistant starch is the sum of starch and products of its degradation

not absorbed in the small intestine of a healthy human (Englyst *et al.*, 1992).

Englyst *et al.* (1992) classified resistant starch into three main types: RS_1: physically inaccessible starch; RS_2: starch of raw (non-gelatinised) granules of some plant species; RS_3: retrograded starch. In successive years, those forms were supplemented with another one, namely RS_4, chemically- or physically-modified starch. RS_4 is obtained by applying standard methods of chemical modification of starch such as crosslinking and substitution (Kapelko *et al.*, 2012a,b; Sang and Seib, 2006; Shin *et al.*, 2004; Wepner *et al.*, 1999; Woo and Seib, 2002; Xie and Liu, 2004; Xie *et al.*, 2006). Similarly, products of starch dextrinisation conducted under specific conditions may be resistant to enzymatic digestion, so some authors also classify them as RS_4. In the case of chemically modified starches, resistance to enzymatic digestion is caused by the formation of steric hindrance at the site of enzymatic action. On the other hand, in the course of starch dextrinisation, 1,2- and 1,3-glucosidic bonds are generated at the expense of 1,4- and 1,6-glycosidic bonds, which are characteristic of starch (Ohkuma *et al.*, 1997; Wang *et al.*, 2001). This is the result of complex processes occurring under the influence of temperature and acid catalysts on starch, including depolymerisation, transglucosidation and repolymerisation. The formation of new bonds makes dextrins less susceptible to the activity of digestive enzymes by reducing the number of targets for potential attack (Cho and Samuel, 2009; Leszczynski, 2004). Thus, starch molecules with α-1,2- and α-1,3-linkages or highly clustered bonds exhibit functional properties similar to those of dietary fibre and prebiotic (Slavin, 2013). A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota (FAO, 2007). Although all prebiotics are fibres, not all fibre is prebiotic (Slavin, 2013).

Over the last decades knowledge on dietary fibre (DF) has increased considerably, both in the physiological and analytical area. Health benefits of DF are associated with bowel function, reduced risk of coronary heart diseases, type 2 diabetes and improved weight maintenance (Westenbrink *et al.*, 2013). The interest in knowing DF and its physiological effects has increased lately and this fact reflects the increased number of scientific publications along the last decades (De Menezes *et al.*, 2013). It is generally recognised that DF is an essential part in the human diet. In our Western society the daily intake of DF is considerably less than the recommended intake of 14.1 g/1000 kcal energy intake for adult. In practice this means an advised daily intake of about 35 g for a man and 28 g for a woman (Brunt and Sanders, 2013). DF is composed by a complex and heterogeneous group of components and can be defined by its physiological characteristics, as well as by its chemical ones. The analytical methods used for DF quantification as a whole or by individual specific components, have been continuously modified (DeVries, 2010; McCleary, 2010). Due to advanced researches on physiological and nutritional properties of specific DF components (fructans, resistant starch, polydextrose and others), several agencies and countries proposed broader definitions, correlated to the physiological effects (AACC, 2001; EC, 2008). The debate on the definition of DF was finalised in 2008/2009 by the Codex Alimentarius Commission defining DF as follows: 'Dietary fibre means carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans' (Codex Alimentarius Commission, 2009).

Traditionally, DF was classified according to its solubility in an attempt to relate physiological effects to chemical types of fibres (Slavin, 2008). Soluble fibres dissolve in water and usually form a gel. Soluble, fermentable fibres have positive physiological effects that may help to improve bowel regularity and result in some health benefits. Soluble fibres are fermented in the large intestine leading to the production of short-chain fatty acids that lower colonic pH and result in a significant prebiotic effect in which the growth of beneficial intestinal microflora (e.g. bifidobacteria, lactobacilli) and faecal glucosidase concentrations are increased, while the growth of pH-sensitive pathogenic bacteria (e.g. clostridia) is prevented or suppressed (Slavin, 2008).

The process of developing new soluble fibre formulations has resulted in increased interest in the products of physical and chemical modification of starch, which could be a source of type 4 resistant starch (RS_4).

The aim of this study was to characterise new dextrins obtained from potato starch by thermolysis in the presence of an inorganic acid (hydrochloric acid) and an organic acid (citric acid). It was hoped that the combination of two factors responsible for starch resistance (temperature and chemical modification) would produce preparations with a higher content of the fraction inaccessible to human digestive enzymes. The study was also designed to explore the possible connections between the method of producing of starch preparations, their structure and their resistance to enzymatic digestion, also with a view to their use as potentially prebiotic substances.

2. Materials and methods

Materials

The materials used in the study included: potato starch, citric acid anhydrous (≥99.5%), heat-stable α-amylase from *Bacillus licheniformis* (cat. no. A3403), protease (cat. no. P3910), amyloglucosidase from *Aspergillus niger* (cat. no. A9913), tris(hydroxyl-methyl)aminomethane (Tris, cat.

no. T1503), 2-morpholinoethanesulfonic acid (MES, cat. no. M3671), celite (cat. no. C8656), salivary alpha-amylase (cat. no. A0521), pepsin (cat. no. P7012), bovine bile (cat. no. B8381), pancreatin (cat. no. P1750), 3,5-dinitrosalicylic acid (DNS, cat. no. D0550) and dextran analytical standards purchased from Sigma-Aldrich (Poznan, Poland); concentrated hydrochloric acid, concentrated sulfuric acid, ethanol (96%, analytical grade), sodium acetate trihydrate, acetic acid (min. 99.5%, analytical grade), benzoic acid (analytical grade), sodium hydroxide (analytical grade), murexide, sodium azide, water for HPLC purchased from POCH (Gliwice, Poland); acetone, sodium sulfate, sodium thiosulfate, potassium iodide, soluble starch, potassium sodium tartrate, potassium hydroxide (analytical grade), boric acid, disodium tetraborate, copper sulfate, sodium chloride (analytical grade), calcium chloride (analytical grade) purchased from Chempur (Piekary Slaskie, Poland).

Preparation of dextrins from potato starch

Potato starch was sprayed with hydrochloric acid solution (0.5%, w/w) to obtain a final HCl concentration of 0.1% dry weight of starch (dws). Citric acid solution (0.5%) was then added to obtain a final organic acid concentration of 0.1% dws. The sample was mixed well and dried at 110 °C to obtain a final moisture content below 5%. The dried sample (10 g) was placed in an anti-pressure bottle (Simax; Kavalierglass Co. Ltd, Praha, Czech Republic), capped and heated at 150 °C for 3 h or 180 °C for 1 h in an ELF 11/6 Eurotherm Carbolite oven (Carbolite, Hope, UK). The product was cooled in a desiccator and milled to a powder. The powdered dextrin was washed with 80% ethanol to remove excess citric acid and low-molecular-weight material formed during dextrinisation, dried at 50 °C overnight and then at 110 °C for 1 h and finally milled in a cyclone laboratory sample mill (UDY Corp., Fort Collins, CO, USA).

Six dextrins were prepared by heating of potato starch: alone at 150 °C for 3 h (Dextrin 0) or 180 °C for 1 h (Dextrin 000); acidified with hydrochloric acid at 150 °C for 3 h (Dextrin 1) or 180 °C for 1 h (Dextrin 111); acidified with both hydrochloric and citric acids at 150 °C for 3 h (Dextrin 2) or 180 °C for 1 h (Dextrin 222).

Determination of reducing sugar content

Reducing sugar content was determined using the DNS method. Briefly, 1.5 ml of DNS reagent (containing DNS, sodium sulfate and sodium hydroxide) was added to 1.5 ml of an aqueous solution of dextrin with a concentration of 2 mg/ml and heated in a boiling water bath for 15 min. (Miller, 1959). Then 0.5 ml of a 40% solution of sodium potassium tartrate was added and, after cooling the sample to room temperature, the absorbance of the solution was measured at a wavelength of 575 nm. The measurement was performed in duplicate. Reducing sugar content was determined from a standard curve based on measurements of the absorbance of standard solutions.

Determination of dextrose equivalent

The value of dextrose equivalent (DE) was evaluated by Schoorl-Regenbogen's method (PN-78/A-74701; Polish Committee for Standardization, 1978). Reducing sugars are determined by reaction of a water soluble portion of the sample with an excess of standard copper sulfate in alkaline tartrate (Fehling's) solution under controlled conditions of time, temperature, reagent concentration and composition, so that the amount of copper reduced is proportional to the amount of reducing sugars in the sample analysed. In this adaptation of Schoorl-Regenbogen's method the reducing sugar concentration expressed as dextrose, is estimated by iodometric determination of the unreduced copper remaining after reaction.

Determination of water solubility and water binding capacity

They were run in water at 20, 60 and 80 °C following the procedure described by Richter *et al.* (1968). A sample (a = 0.5000 g, dry basis) was weighed into 50 ml centrifuge tube of known weight (m_1). Distilled water (35 ml) and stirring bar were then added to the tube. A sample suspension was stirred at 20 °C for 30 min. or heated with stirring in a water bath at 60 or 80 °C for 30 min. After cooling additional 5 ml of distilled water was added and stir bar was removed. The suspension was then centrifuged at 9,000 rpm for 10 min. 10 ml of the supernatant was pippeted into weighing bottle of known weight and dried to constant weight. Solubility (L) was calculated from the equation:

$$L = \frac{(40 \times 100 \times b)}{(10 \times a)} \quad (1)$$

Where a is the sample weight (0.5000 g), b is the weight of residue after drying, volume of evaporated supernatant (10 ml) and total volume of added water (40 ml).

The remaining supernatant was carefully decanted and the centrifuge tube with residue was weighed (m_2). The weight of residue was calculated using the following equation:

$$r = m_2 - m_1 \quad (2)$$

Amount of water (W) bound by the weight (g) of the sample was calculated using equation:

$$W = r - a \quad (3)$$

Where W is the amount of water absorbed by sample, r is the weight of residue in centrifuge tube after decanting the supernatant, a is the sample weight.

Water binding capacity (WBC) as g per g of dry weight was calculated from equation:

$$WBC = W/a \quad (4)$$

pH of aqueous solutions of dextrins

pH of 1% aqueous solutions of dextrins was measured by means of bench pH-meter model HI-223 (Hanna Instruments, Olsztyn, Poland). Measurements were run in triplicates.

Intrinsic viscosity

Intrinsic viscosity (η) was determined using the procedure described by Leach (1963) with an Ubbelohde viscometer (Labit, Stare Babice, Poland) Ubbelohde size 0B (constant K=0.005) at 25 °C. Starch preparations (1 g/100 cm^3) were suspended in 1 M KOH, stirred with a magnetic stir-bar for 30 min, then centrifuged at 9,000 rpm for 10 min. Five concentrations in the range of 0.1-0.5 g/100 cm^3 were used. Intrinsic viscosity was determined by a plot of reduced viscosity vs concentration extrapolated to zero concentration.

Fourier transformation infrared spectra

Fourier transform infrared (FTIR) spectra were recorded in KBr discs using an FTIR Nexus spectrophotometer (Nicolet, Madison, WI, USA) in the region of 4,000-400 cm^{-1} with 32 scans and 4 cm^{-1} resolution.

Determination of degree of substitution

The degree of esterification of starch with citric acid was determined by the method proposed by Klaushofer *et al.* (1979), which is based on the reaction of citric acid and Cu(II) ions resulting in the formation of a stable complex during titration with a solution of copper sulfate. First, 450 mg of dextrin was dissolved in 2 ml of deionised water, then 50 ml of 1 mol/l potassium hydroxide solution was added. The sample was heated in a boiling water bath for 10 min. After cooling to room temperature, the pH of the solution was adjusted to 8.5 with 5 mol/l acetic acid solution. The resulting solution was added to 25 ml of pH 8.5 borate buffer, then 0.3 g of murexide was added and the solution was titrated with 0.05 mol/l copper sulfate solution until the pink/violet colour disappeared. The assay was performed in duplicate. The degree of substitution (DS) was calculated from the formula:

$$DS = 162\ W/100M - (M - 1)W \quad (5)$$

Where W is the percentage of the substituent by weight and M is the molecular mass of the substituent.

Determination of caloric value of dextrins

The calorific value (heat of combustion) of dextrins was measured by bomb calorimeter model KL-12Mn (PRECYZJA-BIT, Bydgoszcz, Poland). The measuring method was compliant with Polish Standard. The measurement consisted in complete combustion of sample in pressurised oxygen atmosphere. The dextrin was placed in a special calorimetric bomb immersed in water. In this way the measurement of water temperature increase was carried out. The heat of combustion was calculated automatically and displayed on computer's monitor. The measuring accuracy of temperature increase was 0.001 °C. The heat energy measured in a bomb calorimeter was expressed either as calories (cal) or Joules (J).

Determination of molecular weight distribution of dextrins by high-performance size exclusion chromatography

The molecular weight distribution of dextrins was determined by high-performance size exclusion chromatography (HPSEC) in a high-performance liquid chromatography/gel permeation chromatography (HPLC/GPC) Smartline system (Knauer, Berlin, Germany) comprising a Knauer Smartline 1000 pump with a Knauer A 1357 injection valve and a RI 2000 detector (SCHAMBECK SFD GmbH, Bad Honnef, Germany). Dextrin (0.02 g) was dissolved in boiling water (10 ml) and stirred for 30 min. in a boiling water bath. The hot sample solution was filtered through a nylon membrane filter (5 μm) prior to injection into the HPSEC system. The final concentration of the dextrin solution filtrate injected (500 μl) was 2 mg/ml. For the separation of fractions, a Shodex OH-pack SB-804 HQ column with a Shodex OH-pack KB-G protective column (Showa Denko KK, Tokyo, Japan) was used. The temperature of the detector was 35 °C. Water for HPLC was used as eluent at a flow rate of 1 ml/min. Dextrans with average molecular weights from 1,270 to 670,000 g/mol were used as standards to determine the molecular weight fraction. The weight-average molecular weight (Mw) and average degree of polymerisation (DP) were determined based on the calibration curve. The percentage of particular fractions detected in the chromatograms was calculated using EuroChrom software (Wissenschaftliche Gerätebau, Berlin, Germany).

Determination of indigestible fraction content in dextrins

Enzymatic-gravimetric AOAC official 991.43 method (AOAC, 2003) was used to analyse the indigestible fraction (IF) content in dextrins. Sample (1.00 g, db) was dispersed in 40 ml of 0.05 M Mes-Tris buffer solution (pH 8.2) in a 400 ml tall-form beaker, and heat stable α-amylase solution (500 U) from *Bacillus licheniformis* was added into it. The beaker was covered with aluminium foil and incubated in a boiling water bath with stirring for 30 min.

The suspension was cooled to 60 °C in a water bath and incubated with protease from *B. licheniformis* (30 units) for 30 min under agitation (120 rpm). Then the suspension was adjusted to pH 4.4-4.6 by adding 0.561 M HCl solution, and amylogucosidase from *Aspergillus niger* solution (600 units) was added. After incubating at 60 °C for 30 min 4 volumes of 95% ethanol (approx. 225 ml, preheated to 60 °C) was added and the mixture was allowed to stand for 1 h at room temperature. The precipitate was filtered on a tared sintered-glass crucible (porosity no. 2) with a bed of 1.0 g of dried Celite as filter aid under vacuum. The solid residue was washed twice with 15 ml of 78% ethanol, twice with 15 ml of absolute ethanol, and once with 15 ml of acetone. The crucible with the residue was dried overnight in an oven at 105 °C, and weighed after cooling to room temperature in a desiccator. IF was calculated as the dried residue divided with the initial weight of dry sample:

% IF = dried residue (g) × 100/initial weight of sample (db, g) (6)

In vitro model of digestion of dextrins in upper gastrointestinal tract

The sample was weighed (1,500±2 mg) into a 100 ml beaker. Distilled water (15 ml) and 0.85% NaCl solution (10 ml) were added to the samples and blanks. The samples were brought to 37 °C with stirring at 250 rpm. All the incubations were performed under the same conditions. Salivary alpha-amylase diluted to 1000 U/ml with 1 mM calcium chloride containing sodium phosphate buffer (20 mM, pH 6.9) was added (50 U/sample). The samples were incubated for 5 min. Hydrochloric acid solution (150 mM) was added and the pH was maintained below 2.5. Pepsin (0.6 mg) dissolved in 1 ml of 20 mM hydrochloric acid per sample was added. Hydrochloric acid (20 mM, 1 ml) was added to the blanks and the samples were incubated for 2 h. Bovine bile (0.4 g) dissolved in 4 ml of 150 mM sodium bicarbonate solution per sample was added to the digest followed by pancreatin (10 mg) dissolved in 4 ml of 150 mM sodium bicarbonate solution per sample. Sodium bicarbonate solution (150 mM, 8 ml) was added to the blanks. The samples were incubated for 3 h at 37 °C with stirring at 250 rpm. The volume of the digest was adjusted to 45 ml in a Falcon tube, centrifuged at 9,000 rpm for 5 min. The supernatant was discarded and the residue was freeze-dried and weighed (Aura *et al.*, 1999).

The dynamics of growth of mixtures of bacteria

This stage of the research examined whether beneficial *Lactobacillus* and *Bifidobacterium* were able to dominate the environment in a common culture with other intestinal bacteria as *Escherichia coli*, *Enterococcus, Clostridium*, *Bacteroides* and *Fusobacterium.* Bacteria were isolated from faeces of 1-year old children and 30- and 70-year old adults. Mixtures of the studied bacterial strains were co-cultured in a growth medium with the addition of dextrin as the only carbon source. The cultures were incubated anaerobically in pH 6.7 at 37 °C for 168 h. Following dilution in physiological salt, the cultures were plated (Koch's plate method) in duplicate immediately after inoculation (0 h) and after 24, 48 and 168 h on selective media: *Lactobacillus* on Rogosa agar (Merck, Darmstadt, Germany), *Bifidobacterium* on reinforced clostridial agar with the addition of the antibiotic dicloxacillin, *E. coli* on ENDO agar (Merck), *Enterococcus* on bile-aesculin agar, *Clostridium* on differential reinforced clostridial medium agar (Merck), *Bacteroides* on Schaedler agar (Biome'rieux, Marcy I'Etoile, France) with an antibiotic gentamicin and *Fusobacterium* on Viande-Levure agar with the addition of vitamin K, hemin and antibiotic streptomycin. The plates were incubated at 37 °C for 48 h; *Lactobacillus*, *E. coli*, and *Enterococcus* under aerobic conditions and *Bifidobacterium*, *Bacteroides* and *Clostridium* under anaerobic conditions in a Ruskinn Concept 400 anaerobic chamber (Biotrace International, Bridgend, UK).

Determination of prebiotic index

Prebiotic fermentation of dextrins were analysed using quantitative equation (prebiotic index; PI). The PI equation is based on the changes in key bacterial groups during fermentation. The bacterial groups incorporated into this PI equation were bifidobacteria, lactobacilli, clostridia and bacteroides. The equation assumes that an increase in the populations of bifidobacteria and/or lactobacilli is a positive effect while an increase in bacteroides and clostridia is negative (Palframan *et al.*, 2003).

3. Results and discussion

An application of both hydrochloric and citric acids led to well water soluble products, of low pH, with increased reducing sugar content and reduced caloric value in comparison to native potato starch.

Water solubility of all samples increased significantly with increasing temperature. WBC of dextrins prepared without any acid was slightly higher that WBC of native potato starch and significantly increased with increasing temperature. An application of hydrochloric and citric acids in dextrinisation process led to products with lower WBC. Dextrinisation of potato starch in the presence of acids resulted in products of very high water solubility at 60 and 80 °C. Therefore, determination of WBC at these temperatures was not possible. The highly soluble dextrins showed relatively high reducing sugar content. It was found that application of both hydrochloric and citric acids led to dextrins with higher reducing sugar content (Table 1). Dextrins prepared with citric acid (2 and 222) exhibited lower solubility in water at 20 °C then dextrins obtained

with only hydrochloric acid. It should result from the formation of cross-linked molecules (Table 1). In order to check if heating of starch with acids resulted in chemical modification (esterification and/or cross-linking) the FTIR were recorded and DS was determined.

However, it is well known that structural changes in the course of dextrinisation generally lead to only a minor response in the infrared-spectral characteristics of dextrins. In the present study the FTIR spectra of dextrins prepared without any acid did not differ from that of native potato starch. The FTIR spectra of hydrochloric or hydrochloric and citric acid-modified dextrins were almost identical to these of dextrins obtained under the same conditions but without any acid. In general, analysis of the FTIR spectra did not demonstrate that the dextrinisation process was accompanied by chemical modification. This was also confirmed by the determination of the degree of substitution of dextrin molecules with citric acid. The DS was 0.008 and 0.006 for Dextrin 2 and 222 respectively, indicating that only about seven out of 1000 hydroxyl groups could be esterified (Table 1). One may conclude that hydrolysis of potato starch with hydrochloric acid considerably dominated chemical modification with citric acid.

In general, the HPSEC profiles of dextrins showed the presence of two fractions: the main fraction with Mw of 3,500 g/mol (average DP=22) and high-molecular-weight fraction with Mw of 2.5×10^6 g/mol (Figure 1). It was found that Dextrin 1, from the heating of potato starch with 0.1% HCl acid at 150 °C for 3 h, contained about 73% of fraction of average DP of 23 (Table 2). The chromatogram recorded for the dextrin prepared by heating of potato starch with 0.1% hydrochloric acid and 0.1% citric acid (Figure 1) showed almost the same molecular weight distribution pattern, as that observed for dextrin prepared without citric acid. However, Dextrin 2 contained 82% of fraction of aver. DP 20 (Table 1). The similar relation was observed for Dextrin 111, prepared without citric acid at 180 °C for 1 h and Dextrin 222, obtained under the same conditions but with citric acid. This is in line with the above findings on the lack or very low degree of chemical modification resulting from heating of potato starch in the presence of citric acid. Intrinsic viscosity is a measure of internal friction or resistance to displacement of high-polymeric molecules in solution. It is related to average molecular size of starch, chain rigidity and branching or shape of the macromolecule (Kim *et al.*, 1995). Intrinsic viscosity values of dextrins prepared with acids ranged from 0.127 to 0.292 (100 cm^3/g) and were more than ten times lower than η values reported by Kim *et al.* (1995) for unmodified potato starch (2.51-3.63).

The dextrinisation of potato starch in the presence of hydrochloric or hydrochloric and citric acids resulted in a significant increase in indigestible fraction content. The enzyme-resistant fraction content in dextrins, determined by AOAC official 991.43 method, reached 7.32% for Dextrin 2 and 6.72 for Dextrin 222 (Table 3). The results of AOAC 991.43 method indicated that highest indigestible fraction content showed samples prepared with citric acid. One can assume that at low hydrochloric and citric acids concentration the repolymerisation of small molecules to form larger, highly-branched molecules took place. In other words, at low acids concentration the rearrangement predominated. It seems that the increase in

Table 1. Physical and chemical characteristics of dextrins.

		Dextrin					
Properties		**0**	**1**	**2**	**000**	**111**	**222**
pH		4.76	4.48	4.11	5.63	4.35	4.01
Water solubility (%)	20 °C	0.40	67.40	29.50	0.16	61.59	54.70
	60 °C	5.55	88.74	63.68	0.88	86.32	89.00
	80 °C	26.81	90.77	94.47	11.95	86.32	89.27
Water binding capacity (g/g)	20 °C	1.78	0.37	1.54	0.97	1.45	1.16
	60 °C	11.88	nd[1]	4.68	13.87	nd	nd
	80 °C	47.63	nd	nd	49.76	nd	nd
Reducing sugar content (%)		3.88	8.65	14.02	3.79	13.90	14.99
Dextrose equivalent		0.14	4.45	5.04	0.17	3.44	3.55
Degree of substitution		-	-	0.008	-	-	0.006
Intrinsic viscosity (100 cm^3/g)		1.927	0.127	0.292	1.642	0.262	0.252
Caloric value (kcal/g)		2.97	2.69	2.83	2.85	2.71	2.83

[1] nd = not determined, preparation was almost completely dissolved.

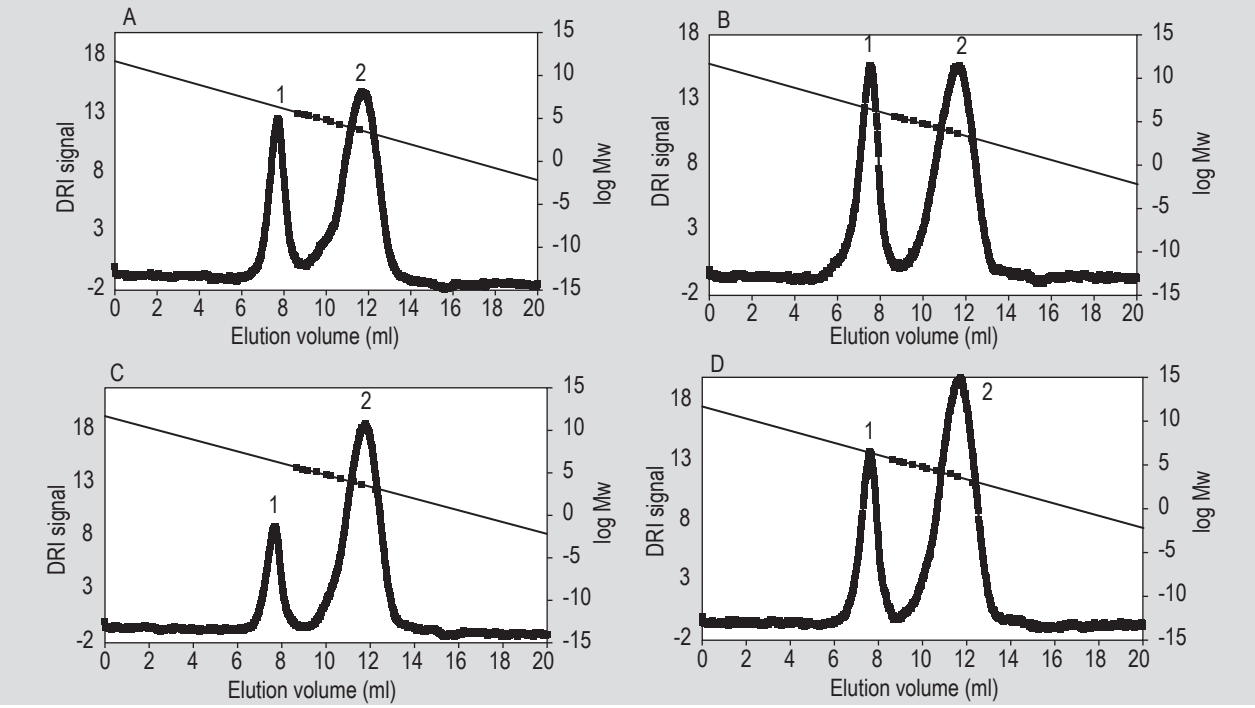

Figure 1. High-performance size exclusion chromatography profiles of (A) dextrin 1, (B) dextrin 111, (C) dextrin 2, and (D) dextrin 222. The straight line is the calibration curve. DRI = differential refractive index; log Mw = weight-average molecular weight.

Table 2. Molecular weight distribution of dextrins[1].

Dextrin	Peak no.	Mw (g/mol)	average DP	% of fraction
Dextrin 1	1	2.12×10^6	13,096	27.31
	2	3.79×10^3	23	72.69
Dextrin 2	1	2.30×10^6	14,183	17.72
	2	3.32×10^3	20	82.28
Dextrin 111	1	2.70×10^6	16,636	33.31
	2	3.69×10^3	23	66.69
Dextrin 222	1	2.49×10^6	15,361	25.05
	2	3.50×10^3	22	74.95

[1] Peak numbers correspond to peaks in Figure 1; Mw = weight-average molecular weight; DP = degree of polymerisation.

Table 3. *In vitro* enzymatic digestion of dextrins (AOAC 991.43 method; AOAC, 2003).

Dextrin	Indigestible fraction content (%)	
	AOAC 991.43 method	*In vitro* model of digestion in upper GI tract
Dextrin 0	5.70	98.21
Dextrin 1	6.75	21.72
Dextrin 2	7.32	20.27
Dextrin 000	5.61	98.11
Dextrin 111	6.23	29.48
Dextrin 222	6.72	24.67

indigestible fraction content in dextrins was probably due to formation of new 1,2- and 1,3-glucosidic bonds between glucoside residues making dextrins less susceptible to the activity of digestive enzymes by reducing the number of targets for potential attack. There is also another possible explanation for the phenomenon of increasing indigestible fraction content in potato starch dextrins prepared with both hydrochloric and citric acids. During heating of potato starch with strong volatile acid a mixture of mainly glucose polymers with mixed glycosidic linkages, called pyrodextrins, is formed. However, dextrinisation products contain also smaller molecules like glucose and/or oligosaccharides. The mixture of starch dextrins,

glucose and another lower order saccharide and additionally catalytic amount of non-volatile citric acid are allowed to react at a temperature range of 150-180 °C to form enzyme-resistant composition. It is believed that citric acid support a condensation reaction of glucose polymers with low molecular weight saccharides to form non-digestible products (Wang and Mungara, 2013). Application of three step *in vitro* enzymatic batch method revealed that indigestible fraction content in dextrins prepared with citric acid was even 25% (Table 3).

In the next step beneficial lactobacilli and bifidobacteria and other intestinal bacteria as *E. coli*, *Enterococcus*, *Clostridium*, *Bacteroides* and *Fusobacterium* isolated from faeces of 1-year old children and 30 and 70-year old adults were co-cultured in the presence of dextrins to examine whether good bacteria can dominate their environment in the presence of a mixture of other also pathogenic bacteria. It was shown that almost all of the tested bacteria were able to grow and utilise dextrins as a source of carbon, albeit to varying degrees. *Lactobacillus*, *Bifidobacterium* and *E. coli* utilised dextrins much better than other bacteria isolated from faeces of 1-year old children. Dextrin 2 and dextrin 222 mostly stimulated the growth of beneficial bacteria and did not contribute to growth of undesirable intestinal microflora (Figure 2).

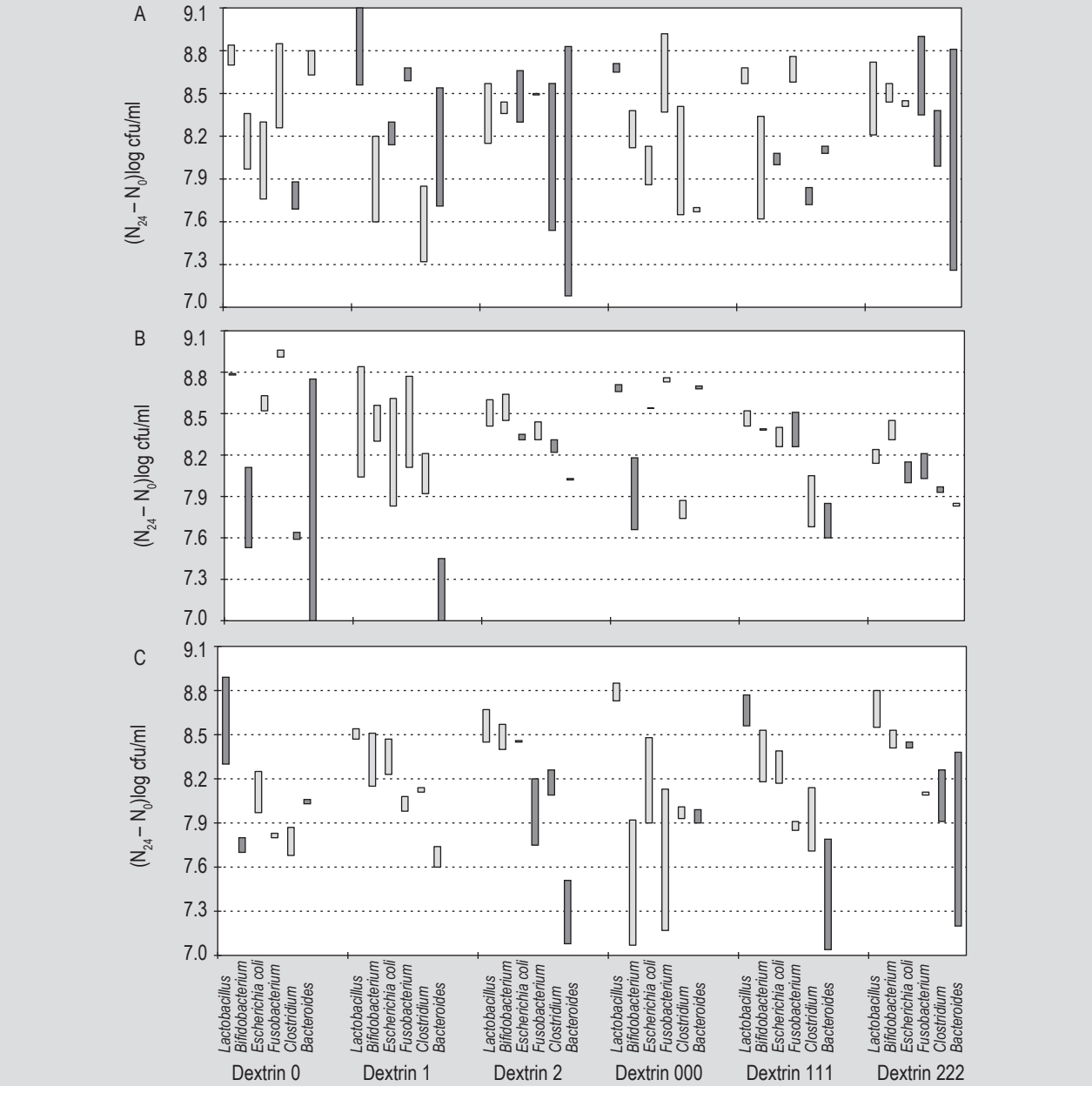

Figure 2. The increase (light) and decrease (dark) in the number of cells ($N_{24} – N_0$) of bacteria isolated from (A) 1-year old children, (B) 30-year old adults, and (C) 70-year old adults and grown together in a medium containing dextrin as the only carbon source.

Analogous results were obtained for isolates from 30- and 70-year old adults. However, the growth of *Lactobacillus* and *Bifidobacterium* was higher for isolates from children and 70-year old adults than for isolates from 30-year old adults (Figure 2).

In conclusion, only dextrins prepared with citric acid ensured growth of good bacteria and simultaneously inhibition of growth of potentially pathogenic bacteria. Other preparations were good carbon source for the majority of strains, however, did not provide selective growth of *Lactobacillus* and *Bifidobacterium* or preparations were not assimilable carbon source and cause dying of most isolates.

Also the PI for dextrins from potato starch was determined. It was found that PI values were always positive and increased with incubation time. It was also found that the prebiotic index was different for different age groups: the highest for 1-year old children and 70-year old adults and the lowest for 30-year old adults.

4. Conclusions

The results showed that the manner of preparation of dextrins from potato starch, in particular the presence of citric acid, had a decisive influence on the chemical structure of dextrins and thus also on their behaviour in relation to digestive enzymes. The studies indicated possibility of preparation of well-soluble, low viscous, low calorie fibre dextrins containing about 80% of molecules with weight average Mw of 3,500 g/mol with increased DF content and increased resistance to digestion and absorption in the upper part of digestive tract. From the present study, it was concluded that *Lactobacillus* and *Bifidobacterium* utilised dextrins much better than other isolated bacteria. Beneficial *Lactobacillus* and *Bifidobacterium*, in the presence of dextrins prepared with citric acid, were able to dominate the environment in the common culture with other bacteria isolated from human faeces. PI values were positive and increased with incubation time. We hope that planned creation of beverages, enriched with soluble fibre dextrins from potato starch with prebiotic properties, will provide many health benefits. However, in order to confirm prebiotic effect of potato starch dextrins further clinical studies are required.

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