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The citric acid-modified, enzyme-resistant dextrin from potato starch as a potential prebiotic

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In the present study, enzyme-resistant dextrin, prepared by heating of potato starch in the presence of hydrochloric (0.1% dsb) and citric (0.1% dsb) acid at 130ºC for 3 h (CA-dextrin), was tested as a source of carbon for probiotic lactobacilli and bifidobacteria cultured with intestinal bacteria isolated from feces of three healthy 70-year old volunteers. The dynamics of growth of bacterial monocultures in broth containing citric acid (CA)-modified dextrin were estimated. It was also investigated whether lactobacilli and bifidobacteria cultured with intestinal bacteria in the presence of resistant dextrin would be able to dominate the intestinal isolates. Prebiotic fermentation of resistant dextrin was analyzed using prebiotic index (PI). In co-cultures of intestinal and probiotic bacteria, the environment was found to be dominated by the probiotic strains of *Bifidobacterium* and *Lactobacillus*, which is a beneficial effect.

Key words: resistant dextrin, prebiotic, intestinal bacteria

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INTRODUCTION

The term prebiotic was first introduced in 1995 by Gibson and Roberfroid, defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson & Roberfroid, 1995). The definition updated by Gibson specifies that a prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Gibson, 2004). The current definition of prebiotics was suggested during the ISAPP experts' meeting in 2008, according to which "a dietary prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (ISAPP, 2008). For a substance to qualify as a prebiotic, it must meet certain criteria: it must be chemically characterized, exhibit health benefits that are measurable and outweigh any adverse effects, and appropriately modulate the composition or activity of the microbiota in the target host (FAO, 2007; Fuentes-Zaragoza *et al*., 2011).

Some carbohydrates, such as fructooligosaccharides (FOS) (Roberfroid *et al*., 2010; Swennen *et al*., 2006), inulin (Roberfroid, 2007; Van Loo, 2006), transgalactooligosaccharides (TOS) (Davis *et al*., 2010), and lactulose (Sekhar *et al*., 2013) are well-accepted prebiotics, while isomaltooligosaccharides (IMO) (Kaneko *et al*., 1995) and xylooligosaccharides (Crittenden and Playne, 2009) are candidate prebiotics. The fermentation of some oligosaccharides is not as selective as that of FOS, so their prebiotic status remains in doubt. Therefore, there is a need for new prebiotic substances that would significantly and selectively stimulate the growth of lactic acid bacteria, while not being fermented, or only slightly so, by other intestinal bacteria, some of which are pathogenic. The search for functional foods or functional food ingredients is undoubtedly one of the leading trends in today's food industry.

Promising sources of prebiotics are starch products, especially resistant starch (RS) (Nugent, 2005), and products of partial degradation of starch, that is dextrins (Betty, 2010; Leszczyński, 2009; Mermelstein, 2009).

The objective of this study was to determine whether dextrin obtained as a result of heating starch in the presence of citric acid (patent claim no. 392895) is a substance with prebiotic properties (Śliżewska *et al*., 2010). Thus, it was examined whether such dextrin would be utilized as a source of carbon by probiotic and intestinal bacteria. It was also investigated whether probiotic lactobacilli and bifidobacteria cultured with intestinal bacteria in the presence of resistant dextrin would be able to dominate the intestinal isolates. In the study, the prebiotic index (PI) and the fermentation products of resistant dextrin were determined.

MATERIALS AND METHODS

Preparation of dextrin. Enzyme-resistant citric acid-modified dextrin (CA–dextrin) was prepared following the method of Kapusniak *et al.* (2008). Thus, potato starch was sprayed with a hydrochloric acid solution (0.5% w/v) to obtain a final HCl concentration of 0.1% on a dry starch basis (dsb). The citric acid solution (0.5% w/v) was then added to obtain a final organic acid concentration of 0.1% dsb. Thoroughly mixed sample was dried at 110ºC to obtain a final moisture content below 5%. Dried sample (10 g) was placed in an anti-pressure bottle (SIMAX), capped and heated at 130ºC for 3 h in an ELF 11/6 EUROTHERM CARBOLITE oven

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Abbreviations: CA–dextrin, enzyme-resistant citric acid-modified dextrin; CFU, colony-forming unit; dsb, dry starch basis; FAO, Food and Agriculture Organization of the United Nations; FOS, fructooligosaccharides; IMO, isomaltooligosaccharides; ISAPP, International Scientific Association of Probiotics and Prebiotics; PI, prebiotic index; RS, resistant starch; TOS, transgalactooligosaccharides

(Hope, England). The product was cooled in a desiccator and milled to powder. Dextrin was then washed with 80% EtOH to remove an excess of citric acid, and low molecular weight material formed during dextrinization, dried overnight at 50ºC, and then at 110ºC for 1 h, and finally milled in a cyclone lab sample mill (UDY Corp., USA).

The dynamics of growth of mixtures of bacteria. The intestinal bacteria *Lactobacillus*, *Bifidobacterium*, *Escherichia coli*, *Enterococcus*, *Clostridium*, and *Bacteroides* were cocultured in the presence of resistant dextrin to determine whether the beneficial bacteria *Lactobacillus* and *Bifidobacterium* can dominate their environment in the presence of a mixture of isolated intestinal bacteria. Inoculants of bacterial monocultures were prepared in such a way that after 24 h of growth the number of particular bacteria ranged from 3.50×10^7 to 4.10×10^7 CFU/mL, corresponding to the number of these bacteria in the terminal section of the ileum (Ouwehand and Vesterlund, 2003). The monocultures of bacteria isolated from three 70-year-old persons were incubated in liquid MRS (*Lactobacillus* and *Bifidobacterium*), in liquid VL medium (*Clostridium* and *Bacteroides*) and in liquid broth (*Escherichia coli* and *Enterococcus*). All monocultures were incubated in sterile 15 mL test tubes (Marfour) — *Lactobacillus, Escherichia coli, Enterococcus* under aerobic conditions and *Bifidobacterium, Bacteroides* and *Clostridium* under anaerobic conditions. After incubation, the cultures were centrifuged in a MPW-350R centrifuge (Med. Instruments, Poland) at 9000 rpm for 10 min at 22°C, the supernatant was decanted and the biomass was transferred to 100 mL of Wynne *et al.* (2004) medium with the addition of resistant dextrin (bile salts, 0.5 g/l; NaCl, 0.1 g/l; K_2HPO_4, 0.04 g/l; KH_2PO_4, 0.04 g/l; L-cysteine, 0.5 g/l; $MgSO_4x7H_2O$, 0.01 g/l; $NaHCO_3$, 0.39 g/l; Tween, 2 g/l; peptone K, 10 g/l; $MnSO_4x4H_2O$, 0.01 g/l; hemin, 0.05 g/l; Vitamin K, 0.01 g/l; resistant dextrin, 10 g/l). The cultures were incubated for 168 h under anaerobic conditions (similar conditions as in the intestine). Following dilution in physiological salt, the cultures were plated (Koch's plate method) in duplicate immediately after inoculation (0 h) and after 24, 48, 72 and 168 h on selective media: *Lactobacillus* on Rogosa agar, *Bifidobacterium* on RCA agar with the addition of the antibiotic dicloxacillin, *Escherichia coli* on ENDO agar, *Enterococcus* on bile-aesculin agar, *Clostridium* on DRCM agar and *Bacteroides* on Schaedler agar with an antibiotic. The plates were incubated for 48 h at 37°C; *Lactobacillus, Escherichia coli*, and *Enterococcus* under aerobic conditions and *Bifidobacterium, Bacteroides* and *Clostridium* under anaerobic conditions in a Concept 400 anaerobic chamber (Ruskinn Biotrace, USA).

Determination of prebiotic index (PI). Prebiotic fermentation of resistant dextrins was analyzed using a 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).

The PI equation is described below:

PI = (Bifidobacterium/Total bacteria) — (Bacteroides/Total bacteria) + (Lactobacillus/Total bacteria) – (Clostridium/Total bacteria).

pH changes. Changes in pH were monitored with an Elmetron CP-401 pH-meter (Elmetron, Poland).

RESULTS AND DISCUSSION

It seems likely that prebiotic activity will be exhibited by dextrin obtained by simultaneous thermolysis and chemical modification of potato starch in the presence of a volatile inorganic acid (hydrochloric acid) as a catalyst of the dextrinization process and an excess amount of an organic acid (citric acid) as a modifying factor (patent claim no. 392895 "Preparation with prebiotic qualities"). The enzyme-resistant dextrin, prepared by heating of potato starch in the presence of hydrochloric (0.1% dsb) and citric (0.1% dsb) acid at 130°C for 3 h (CA-dextrin), was tested as a source of carbon for probiotic lactobacilli and bifidobacteria cultured with the intestinal bacteria isolated from the feces of three healthy 70-year-old volunteers.

In media where CA-dextrin was the source of carbon, all *Lactobacillus* and *Bifidobacterium* strains reached the stationary phase at 24 h of incubation. The number of bacteria of the genus *Lactobacillus* and *Bifidobacterium* in the stationary phase was similar and amounted to: 8.59 log CFU/mL and 8.42 CFU/mL, respectively. At 168 h of culture in a medium with dextrin modified with citric

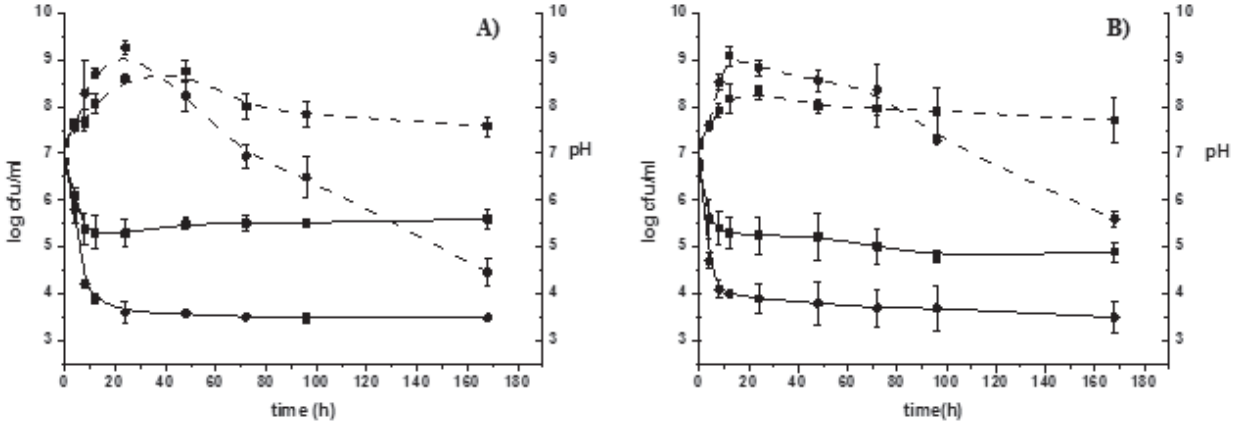

Figure 1. The growth curves (--) and changes in pH (—) for *Lactobacillus* (A) and *Bifidobacterium* (B) bacteria grown in the medium containing CA–dextrin (■) or glucose (control) (●).
Results show means and standard deviations of n = 3 replicates.

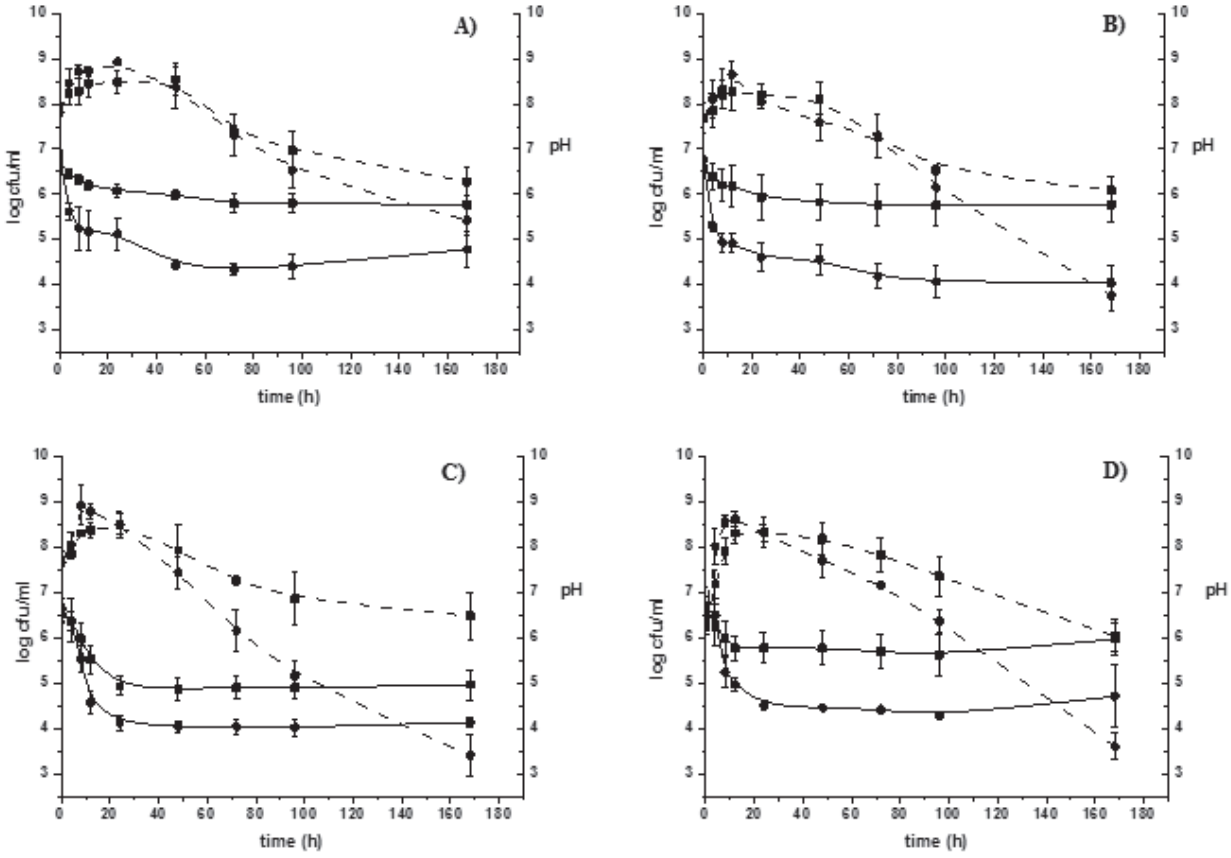

Figure 2. The growth curves (--)and changes in pH (—) for *Bacteroides* (A), *Clostridium* (B), *Enterococcus* (C) and *Escherichia coli* (D) bacteria grown in the medium containing CA–dextrin (■) or glucose (control) (●).
Results show means and standard deviations of n = 3 replicates.

-acid, the number of lactobacilli and bifidobacteria remained high and ranged from 7.61 to 7.89 log CFU/mL, which shows their substantial viability (Fig. 1).

Control strains were grown in media with glucose. At 24 h of incubation, in cultures with glucose the number of lactobacilli and bifidobacteria amounted from 9.15 to 9.40 log CFU/mL. The bacteria entered the stationary phase, similarly as in media containing dextrin, after 24 h of incubation. However, the stationary phase lasted much shorter than in media containing dextrin. At 168 h of culture, the number of viable *Lactobacillus* and *Bifidobacterium* cells cultured with glucose was much lower than that of cells cultured with resistant dextrin, and amounted from 4.52 to 5.75 log CFU/mL (Fig. 1).

In the medium containing dextrin, the acidifying activity of bifidobacteria was higher than that of lactobacilli. After incubation, a test of culture pH revealed that *Bifidobacterium* had the highest acidifying activity (pH 4.8), while *Lactobacillus* — the lowest (pH 5.6). In the control medium containing glucose, the pH of *Lactobacillus* and *Bifidobacterium* cultures decreased much more than that in the medium containing dextrin; at 168 h the pH was 3.50 (Fig. 1).

In media containing CA-dextrin, the other bacteria isolated from human feces were able to grow, but the degree of dextrin utilization depended on the strain. In media with resistant dextrin, bacteria entered the stationary phase between 12 and 48 h of incubation and it lasted for 20–30 consecutive hours. In this phase, the highest cell count was found for *Bacteroides* strains (8.50 log CFU/mL) (Fig. 2). Also *Enterococcus* strains grew successfully, with the number of cells in the stationary phase reaching 8.48 log CFU/mL. Lower growth was found for *Escherichia coli* and *Clostridium* strains (8.30 and 8.25 log CFU/mL, respectively) (Fig. 3). In media containing dextrin, bacteria isolated from human feces preserved high viability, and at 168 h the number of viable cells was larger by 1–2.5 log cycles than that of the control cells grown with glucose.

Out of the bacteria isolated from human feces, the most acidifying ones were *Enterococcus* strains, which decreased pH to about 4.90. In cultures containing dex-

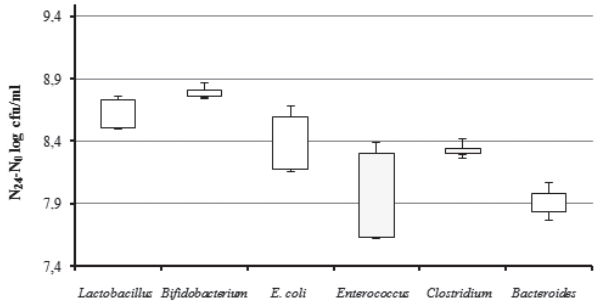

Figure 3. The increase in the number of cells intestinal bacteria (N_{24}–N_0) grown together for 24 h in the medium containing CA–dextrin.
N_{24} — the number of bacterial cells at 24 h of incubaction
N_0 — the number of bacterial cells at incubaction. Results show means and strandard deviations of n = 3 replicates.

trin, no significant differences were found between the remaining strains, as the pH ranged from 5.70 to 6.20. *Enterococcus* strains were most active by 24 h of incubation, while the others by 12 h of incubation; later on the pH values did not change significantly (Fig. 2).

It was shown that all the bacteria isolated from human feces were able to grow and utilize CA-dextrin as a source of carbon, albeit to varying degrees. The highest growth was recorded for *Lactobacillus* and *Bifidobacterium*. *Bifidobacterium* strains were also characterized by the highest acidifying activity (lowering pH to 4.8), which remains consistent with the results reported by other authors (Bielecka *et al*., 2002; Crittenden *et al*., 2001). The weakest growth was observed for *Clostridium* and *Escherichia coli*. It was found that the stationary phase for *Lactobacillus* and *Bifidobacterium* strains was much longer than for other intestinal bacteria. After prolonging the culture time to 72–168 h, which corresponds to a retarded or pathological passage of digesta through the large intestine, the viability of intestinal bacteria in a medium with resistant dextrin was found to be lower by 1–1.5 log cycles than that of *Lactobacillus* and *Bifidobacterium*. The number of *Lactobacillus*, *Bifidobacterium*, and other bacteria isolated from fecal samples grown in the media containing 1% glucose was lower by 1–2.5 log cycles than that of corresponding bacteria grown in a medium containing dextrin. This may have been caused by lower pH values of the controls, under which the culture environment became unfavorable to preserving high viability by the studied bacteria. This may have been also caused by the protective effects of dextrin on the bacteria.

The objective of the study was to determine whether lactobacilli and bifidobacteria co-cultured in the presence of CA-dextrin would be able to dominate over the intestinal bacteria such as *E. coli*, *Enterococcus*, *Clostridium* and *Bacteroides*. The bacteria were cultured anaerobically at pH 6.7, under conditions similar to those in the large intestine, using a 10^7 CFU/mL bacterial inoculum and a culture time of up to 168 h.

It was shown that after 24 h of incubation strains isolated from the feces of 70-year-old subjects were dominated by *Lactobacillus* and *Bifidobacterium*. The number of bacterial cells (N_{24}–N_0) at 24 h amounted to 8.50–8.70 log CFU/mL by *Lactobacillus* and 8.70-8.80 log CFU/mL by *Bifidobacterium* (Fig. 3). *Escherichia coli* was also found to grow well (the number of cells increased to 8.20-8.60 log CFU/mL). The growth of *Enterococcus* and *Clostridium* strains amounted respectively to 7.60–8.30 log CFU/mL by *Enterococcus* and 8.30 log CFU/mL by *Clostridium*. There was no increase in the number of cells in *Bacteroides* strains and after 24 hours of incubation, the number of bacterial cells was at the level of inoculation (Fig. 3).

It was found that higher counts of the studied probiotic bacteria and of bacteria isolated from fecal samples may have been caused by interactions taking place in the mixture, such as multi-level protocooperation or metabiosis, where strains supply each other with nutrients, produce growth substances, or exchange gases. It is also possible that syntrophy occurred, whereby protons were used as electron acceptors. Such fermentation may have led to the production of molecular hydrogen, which could have been transferred from one species of microorganisms to another (Dale and Park, 2010; Ouwehand and Vesterlund, 2003).

It was shown that the PI values in media with CA-dextrin were positive; furthermore, the prebiotic index increased with the time of culture (from 0.033 at 24 h of incubation to 0.176 at 168 h), which proves that beneficial bacteria (*Bifidobacterium* and *Lactobacillus*) can dominate their environment in the presence of a mixture of intestinal bacteria cultured with the addition of resistant dextrin.

The calculated PI values for CA-dextrin were higher than those reported by Olano-Martin *et al*. (2003) or by Kordyl (2010) for inulin and oligosaccharides under the same incubation conditions (anaerobiosis; 1% prebiotic addition; pH 6.8; incubation temperature of 37°C), which shows that CA-dextrin may act as a prebiotic substance.

CONCLUSIONS

The experiments showed that dextrin obtained as a result of heating potato starch in the presence of hydrochloric acid (0.1% of starch dry mass) and citric acid (0.1% of starch dry mass) at 130ºC for 3 h specifically supported growth of probiotic strains of *Bifidobacterium* and *Lactobacillus*. However, in order to recognize a potential prebiotic effect of citric acid – modified dextrin additional *in vivo* studies are required.

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