Prebiotic properties of potato starch dextrins

Właściwości prebiotyczne dekstryn otrzymanych ze skrobi ziemniaczanej

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Summary

The objective of the present study was to compare the prebiotic properties of starch dextrins, that is, resistant dextrins obtained from potato starch in the process of simultaneous thermolysis and chemical modification, which were selected based on previous research. Both prepared dextrins met the definition criterion of dietary fiber and also the basic prebiotic criterion – they were not degraded by the digestive enzymes of the initial sections of the gastrointestinal tract. The growth of probiotic lactobacilli and bifidobacteria, as well as *Escherichia coli*, *Enterococcus*, *Bacteroides*, and *Clostridium* strains isolated from feces of healthy people, showed that both studied dextrins were utilized as a source of assimilable carbon and energy by the strains. Furthermore, better growth (higher numbers of cells) counts of probiotic bacteria than those of fecal isolates indicated that the studied resistant dextrins showed a selective effect. Both dextrins might be considered as substances with prebiotic properties due to their chemical and physical properties and selectivity towards the studied probiotic bacterial strains.

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The past several years have witnessed dynamic growth of the functional food market and increasing consumer awareness of food quality. There has been rising interest in products enriched with fortifying substances such as dietary fiber and prebiotics.

Dietary fiber consists of carbohydrate polymers containing 10 or more monomers which are not hydrolyzed by endogenous enzymes in the human small intestine and which belong to one of the following three groups: a) edible carbohydrate polymers naturally occurring in food and consumed with it; b) carbohydrate polymers which may be obtained from organic materials by physical, enzymatic, or chemical methods and which are beneficial for human health; c) synthetic carbohydrate polymers which are beneficial for human health [6].

Prebiotics are non-viable food ingredients which confer health benefits on the host as a result of modulation of the composition of gastrointestinal microflora [11]. Dietary fiber also includes that part of starch that is not completely digested over 120 min and is transferred from the small intestine to the large intestine in undecomposed or partly hydrolyzed form. This part of starch is defined as resistant starch. In other words, resistant starch consists of starch and the products of its degradation that are not digested and absorbed in the small intestine of healthy individuals [10]. Resistant starch occurs in 4 different forms (RS1, RS2, RS3, and RS4), although some publications have described a fifth type of resistant starch (amylose-lipid complexes) [5,9,10,14,15,16,27,39]. RS4 consists of the products of chemical or physical (thermal) modification of starch, including simultaneous chemical and physical modification. It is well known that heating starch with acids, which act as catalysts, leads to starch dextrinization, and dextrins obtained under appropriate conditions may exhibit properties characteristic of resistant starch. The products of thermal starch depolymerization are resistant to amylolytic enzymes due to the changes in the structure of starch caused by heating, involving depolymerization, transglycosylation, and repolymerization of starch fragments. An increased time of dextrinization results in a greater number of 1,2- and 1,3-glycosidic bonds between anhydroglucose units. The newly formed molecules with α-1,2- and α-1,3- bonds are not hydrolyzed by the enzymes present in the gastrointestinal tract, and so they show functional properties similar to those expected of dietary fiber components or, under certain conditions, prebiotics [15,19,20,30,38,40].

In this study, potato starch was used to produce potentially prebiotic preparations. It was subjected to simultaneous thermolysis and chemical modification in the presence of a volatile inorganic acid (hydrochloric acid), acting as a catalyst in the process of dextrinization, and an organic acid (citric or tartaric acid) as a modifier [18,21,22].

An important factor in the production of potentially prebiotic preparations is the relationship between the content of the undigested fraction and water solubility [25]. It has been shown that pyrodextrins containing a greater proportion of the undigested fraction are less readily soluble in water, which may be due to repolymerization or the formation of non-starch compounds. In turn, Kapusniaka et al. [21,22] reported that heating potato starch without acid at 130°C for 180 min led to a low-solubility product (2.7%), while heating starch in the presence of hydrochloric acid considerably improved solubility (up to about 67%). This was caused by the hydrolysis of glycosidic bonds and the formation of shorter glucan chains, or even oligosaccharides and simple sugars. An additional treatment with citric acid led to a dextrin with 63% solubility. The water solubility of the dextrin obtained in the presence of tartaric acid was approximately 68% [1,18,21,22]. It was found that the mean molecular weight (M_w) of the dextrin produced using tartaric acid was 1828 g mol^{-1} (DP 11 on average), while that of the dextrin produced using citric acid was 4.8×10^3 g mol^{-1} (DP 25-30 on average) [1,18]. Thus both substances met the definition criterion of dietary fiber.

However, from the point of view of resistance to amylolytic enzymes in the gastrointestinal tract, of essence is not only the molecular weight of a substance, but also its chemical structure. Particularly important are the number and type of branches in the molecules. According to a study using high performance anion exchange chromatography (HPAEC), the mean length of dextrin chains is smaller than the mean DP of the main fraction, which proves the presence of branches in dextrins [1,18].

The dextrinization of starch in the presence of citric and tartaric acids led to an increase in the undigested fraction. The total content of dietary fiber determined by the AOAC 2001.03 method was approximately 30% for the dextrin obtained using citric acid and 50% for the dextrin modified with tartaric acid.

In turn, the enzymatic-spectrophotometric (Englyst) method showed that the actual content of the undigested fraction in dextrins was much higher, up to 70% [1,18]. Based on enzymatic tests, it was postulated that the dextrin obtained in the presence of excess tartaric acid may be classified as RS4 starch. This was also confirmed by previous studies, which indicated that heating potato starch in the presence of tartaric acid led to a high degree of chemical modification [1,18]. In turn, in the case of dextrin obtained in the presence of citric acid, hydrolysis induced by hydrochloric acid largely dominated chemical modification with citric acid. This was confirmed both by previous Fourier transform infrared spectroscopy (FTIR) tests and by determining the degree of substitution (DS) of dextrin molecules by citric acid (0.0073%), which means that only about 7 hydroxy groups out of 1000 could be esterified [1,21]. Thus, dextrins obtained from potato starch met the basic prebiotic criterion – they were not degraded by the digestive enzymes of the upper sections of the gastrointestinal tract.

A preliminary microbiological study [1,2] investigated whether resistant dextrins obtained by heating starch with hydrochloric and citric acids at 130°C for 3 h and with hydrochloric and tartaric acids at 130°C for 2 h may serve as a source of carbon for pure cultures of selected probiotic bacteria: *Lactobacillus casei* DN-114 001, *Lactobacillus casei* Shirota, *Lactobacillus rhamnosus*, *Bifidobacterium animalis* DN-173 010, and *Bifidobacterium bifidum* Bb12. Another study tested the ability of *Bacteroides*, *Clostridium*, *Escherichia coli*, and *Enterococcus* strains isolated from the feces of 30-year-olds with stable intestinal microflora to use resistant dextrins [1,2].

It was found that resistant dextrins were used as a source of carbon by strains with confirmed probiotic properties. It was also found that the intestinal strains isolated from the feces of 30-year-olds also utilized resistant dextrins as a source of carbon, but to a much lesser extent than the probiotic strains. In the stationary phase, the number of probiotic lactobacilli and bifidobacteria in media containing dextrins obtained using citric and tartaric acids was higher by an order of magnitude than the number of the intestinal strains. At the end of the culture period (168 h), the probiotic strains were found to dominate over the intestinal isolates.

The objective of the present study was to compare the prebiotic properties of starch preparations, that is, resistant dextrins obtained from potato starch in the process of simultaneous thermolysis and chemical modification, which were selected based on previous research[20]. We investigated the influence of resistant dextrins on bacterial strains with proven probiotic properties as well as on bacterial strains isolated from the feces of children and adults. The goal was to determine whether the process of production of starch preparations had an impact on their prebiotic properties and whether these preparations were used in the same way by bacterial isolates from the feces of people of very different ages (1- and 8-year-old children and 30-year-old adults).

MATERIALS AND METHODS

Resistant dextrins

The dextrins were prepared by heating potato starch with hydrochloric and citric acids (D1) at 130°C for 240 min and with hydrochloric and tartaric acids (D2) at 130°C for 120 min [22]. The physical and chemical properties of the dextrins were presented previously [1,18].

The content of the resistant fraction in the studied dextrins was determined using the official AOAC 2001.03 method as well as the Englyst method [1,18].

Bacterial cultures

Probiotic bacteria – *Lactobacillus casei* DN-114 001, *Lactobacillus casei* Shirota, *Lactobacillus rhamnosus* Lakcid, *Bifidobacterium animalis* DN-173 010, *Bifidobacterium bifidum*

Bb12 – were isolated from food products and a pharmaceutical product. *Bacteroides, Clostridium*, *Escherichia coli* and *Enterococcus* strains were isolated from feces of three healthy children aged one year and eight years and three 30-year-old male volunteers (total of 36 strains) [24].

Prior to experiments bacteria were activated by twofold inoculation (3%): probiotic bacteria in liquid deMan, Rogosa and Sharpe (MRS) broth, *Bacteroides* and *Clostridium* in liquid Viande Levure (VL) broth, and *Escherichia* and *Enterococcus* in nutrient bouillon. All broths were purchased from BTL (Lodz, Poland).

Probiotic and intestinal bacteria were cultured in media according to Wynne et al. (2004) [41], with the source of carbon being resistant dextrins at a concentration of 1%. The media were inoculated with 3% inoculum of the studied bacteria grown in pure cultures. The controls were cultures of the bacteria in media containing 1% glucose. The strains were cultured up to 168 h.

Growth of the bacteria was estimated by the plate method after 4, 8, 12, 24, 48, 72, 96, and 168 h of incubation using the following media: MRS for *Lactobacillus*, reinforced clostridial agar (RCA) [8] with the addition of the antibiotic dicloxacillin for *Bifidobacterium*, MacConkey medium for *Escherichia coli* (MERCK, Darmstadt, Germany), agar medium with bile and aesculin for *Enterococcus* (MERCK, Darmstadt, Germany), and VL medium for *Clostridium* and *Bacteroides*. The plates were incubated for 48 h at 37°C.

Co-cultures of probiotic bacteria and bacteria isolated from the feces of people at different ages were inoculated with the studied bacteria in such a way that the number of cells of particular strains ranged from 3.2×10^7 to 4.5×10^7 CFU mL^{-1} [33]. The controls consisted of cultures of the same bacteria in media without saccharides. Co-cultures were conducted under anaerobic conditions at pH 6.8 and 37°C.

The numbers of probiotic and intestinal bacteria were determined similarly as for pure cultures, but using different media: Rogosa medium for *Lactobacillus* (MERCK, Darmstadt, Germany), RCA with an addition of dicloxacillin for *Bifidobacterium*, ENDO for *Escherichia coli* (MERCK, Darmstadt, Germany), medium with bile and aesculin for *Enterococcus* (MERCK, Darmstadt, Germany), differential reinforced clostridial broth (DRCM, MERCK, Darmstadt, Germany), and Schaedler medium with the antibiotic gentamycin (BioMerieux, Marcy I'Etoile, France).

Growth of bacteria was determined as follows:

$N = N_k - N_0$, where:

N is the increase in the number cells;

N_k is the number of cells in the stationary phase (CFU mL^{-1});

N_0 is the number of cells introduced into the culture medium (inoculum) (CFU mL^{-1}).

Determination of fermentation products by high performance liquid chromatography (HPLC)

Fermentation products were determined using the Thermo/Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA, USA) equipped with refractive index (RI) and ultraviolet (UV) detectors. Analyses were performed using a Bio-Rad AMINEX HPX-87H (300x7.8 mm) column (Hercules, CA, USA). Operation conditions were as follows: mobile phase: 0.005 M H$_2$SO$_4$; flow rate: 0.6 ml min^{-1}; column temperature: 60°C. Samples were microfiltered using 0.22 lm syringe filters prior to injection on the HPLC system. Quantification of fermentation products was carried out using the external standard method. Lactic acid, formic acid, acetic acid, propionic acid, butyric acid, succinic acid, ethanol and acetaldehyde of known retention times were used as external standards. All solutions were filtered through 0.22 lm syringe filters, and injected into the HPLC system to provide standard curves (concentration versus peak area), and for calculating the quantities of products (organic acids, aldehydes and ethanol). Linear regression curves based on peak areas were calculated for the individual standards covering a broad range of concentrations.

Determination of prebiotic index (PI)

The prebiotic index (PI) was analyzed using the quantitative equation [34]

PI = (Bif/Total) – (Bac/Total) + (Lac/Total) – (Clos/Total)

where:

Bif = number of *Bifidobacterium* cells at sample time/number at inoculation;

Bac = number of *Bacteroides* cells at sample time/number at inoculation;

Lac = number of *Lactobacillus* cells at sample time/number at inoculation;

Clos = number of *Clostridium* cells at sample time/number at inoculation;

Total = number of bacteria cells at sample time/number at inoculation (the sum of *Bacteroides*, *Clostridium*, *Lactobacillus*, *Bifidobacterium* bacteria).

Results and discussion

Growth of probiotic and intestinal strains in the presence of resistant dextrins (pure cultures)

Both probiotic and intestinal strains used resistant dextrins as a source of carbon and energy. The highest cell counts were found for the strains *Lactobacillus rhamnosus* Lakcid (5.63 × 10^8 CFU mL^{-1}) and *Bifidobacterium bifidum* Bb12 (4.08 ×10^8 CFU mL^{-1}).

Probiotic bacteria utilized dextrin obtained in the presence of tartaric acid (D2) as a source of carbon in a more efficient way than when obtained in the presence of citric acid (D1), which was reflected by the higher cell counts at 24 h of incubation with D2 (Fig. 1). The number of probiotic bacteria cells in the control cultures was 6% to 50% higher than that in cultures with resistant dextrins (Fig. 1).

The growth of fecal isolates depended on the age of the person from whom the strain was isolated and the type of dextrin. *Escherichia coli* grew better; its cell count increased by 1.14 to 1.9 log CFU mL^{-1} and was higher in the culture with dextrin D2 (Fig. 2a). Resistant dextrins were used least efficiently by *Enterococcus* strains, except
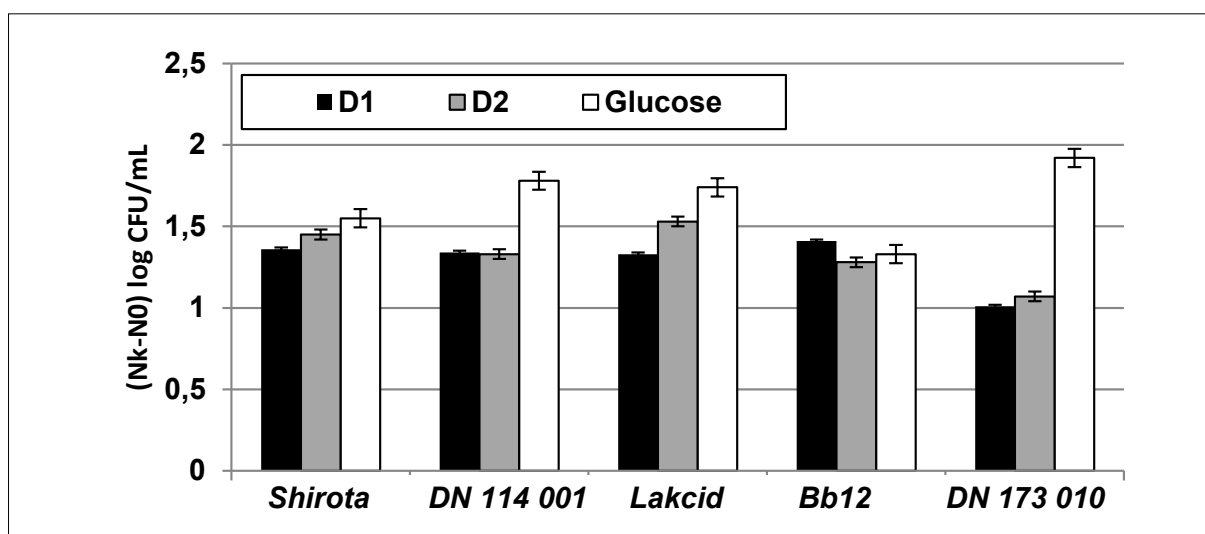

Fig. 1. The increase in the number of cells (N_k–N_o) of probiotic bacteria at 24 h of cultivation in medium with dextrin D1, D2 and in medium with glucose
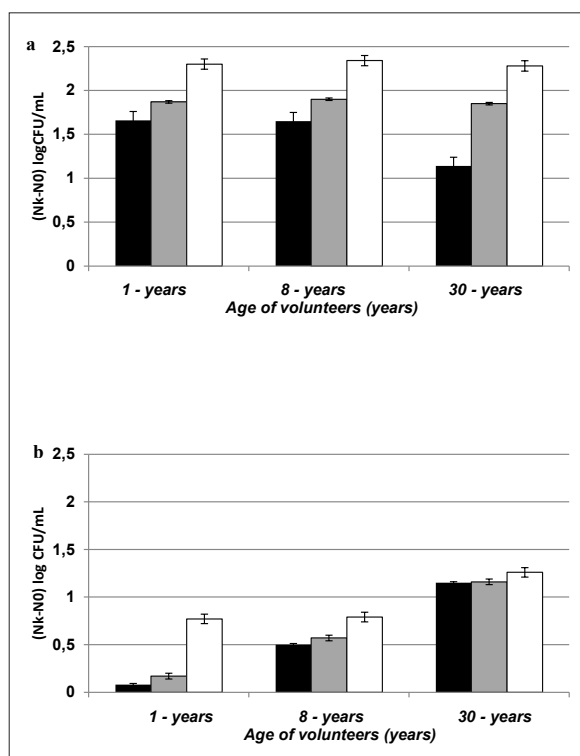
Fig. 2. The increase in the number of cells (N_k–N_o) of bacteria isolated from feces of persons aged 1, 8 and 30 years at 24 h of cultivation in medium with dextrin D1, D2 and in medium with glucose a) *Escherichia coli; b) Enterococcus, c) Clostridium, d) Bacteroides*

for the isolates from 30-year-olds (Fig. 2b). In turn, the increase in the cell counts of *Clostridium* and *Bacteroides* strains was higher by about 5% to 15% in cultures with dextrin D1 than in those with D2 (Fig. 2c,d). In media containing dextrins (D1, D2), the number of cells of all the studied strains was slightly lower than in control cultures with glucose (Fig. 2a,b,c,d).

The dextrins used in the study consisted of approximately 70% resistant fraction and had branched molecules, so they contained bonds other than the typical a-(1→4)-glycosidic bonds occurring in starch [18]. According to the literature [31], commercially available maltodextrin (Fibersol) is obtained from corn starch by pyrolysis followed by enzymatic hydrolysis to replace the a-(1→4)-glycosidic bonds typical of starch with (1→2)- and (1→3)- a and b glycosidic bonds. The obtained product with the new types of bonds is not metabolized in the upper part of the gastrointestinal tract and is transferred to the colon, where it serves as a source of carbon and energy for the intestinal bacteria occurring there. The presence of such bonds has also been found in another substance having the properties of soluble fiber, that is, Nutriose FB, which is obtained from wheat starch. It contains approximately 13% a and b (1→2)-glycosidic bonds and 14% a and b (1→3)-glycosidic bonds [36]. It has been shown that Fibersol stimulates the growth of beneficial intestinal flora including those stra-

ins that are therapeutically administered as probiotics. At the same time, this preparation is not a good source of carbon for growth of undesirable bacteria [17,28]. Nutriose promotes the growth of saccharolytic bacteria, at the same time inhibiting pathogenic *Clostridium* bacteria in the human colon [17,26].

Similar findings were made in the present study for potato starch dextrins: all the tested bacteria, both probiotic ones and those isolated from human feces, were able to utilize resistant dextrins for growth, albeit to a different extent. Irrespective of the source of carbon, the probiotic *Lactobacillus* and *Bifidobacterium* strains grew the best (the average cell count was 10^8 CFU mL^{-1}), which is consistent with the results reported by other authors [3,7,35]. The weakest growth was observed for *Clostridium* and *Enterococcus* (the mean cell count was 10^7–10^8 CFU mL^{-1}). After prolonging culture time to 72–168 h, which corresponds to retarded or pathological passage of large intestine contents, the number of intestinal bacteria in media with resistant dextrins was of one or two orders of magnitude lower than that of probiotic bacteria. In comparison to the cell counts in the control medium with 1% glucose, the number of probiotic bacteria and those isolated from human feces in media with dextrins modified by either citric or tartaric acids was two to three orders of magnitude higher. This may have resulted from the protective effects of dextrins on bacteria.

The age of the persons from whose feces bacteria were isolated had an influence on bacterial growth in medium with dextrin. Bacteria isolated from the feces of 30-year-olds grew better than those from the feces of 30-year-olds. This may have been caused by differences in the species composition of microflora in the last part of the gastrointestinal tract of people of different ages.

PRODUCTS OF RESISTANT DEXTRIN FERMENTATION GENERATED BY PROBIOTIC BACTERIA

All probiotic *Lactobacillus* and *Bifidobacterium* strains produced lactic, acetic, and propionic acids as a result of fermentation of resistant dextrins.

Lactobacilli mostly produced lactic acid. The concentration of this acid varied and, depending on the strain and type of dextrin, ranged from 52.2 to 110.9 mg 100 mL^{-1} (Table 1). The greatest amount (110.9 mg 100 mL^{-1}) was produced by *Lactobacillus* strains as a result of fermentation of dextrin D2 (Table 1). In turn, bacteria fermenting dextrin D1 produced 27% to 34% less lactic acid, except for *Lactobacillus rhamnosus* Shirota, which produced 50% less lactic acid (Table 1). Probiotic lactobacillus strains also produced acetic acid, but not as much as bifidobacteria (48% to 65% less).

Bifidobacterium strains produced lactic acid, at a rate of 75.5 to 109.3 mg 100 mL^{-1}. More lactic acid was produced from dextrin D2 than D1. Bifidobacteria also produced a considerable amount of acetic acid (45–48.4 mg mL^{-1}), especially

as the fermentation product of dextrin D1 (Table 1). *Bifidobacterium* was the only bacterial species to produce formic acid, whose concentration was similar for both dextrins and ranged from 30.2 to 50.2 mg 100 mL^{-1} (Table 1).

All probiotic strains produced propionic acid, but irrespective of the strain and dextrin, the concentration of this acid was low and ranged from 2.0 to 5.0 mg 100 mL^{-1} (Table 1).

It was found that all probiotic bacteria fermenting dextrin D1 generated 46% to 70% less lactic acid than in the process of glucose fermentation (control cultures). However, the quantities of lactic acid produced by *Lactobacillus rhamnosus* Shirota, *Lactobacillus casei* DN 114 001, *Bifidobacterium bifidum* Bb12, and *Bifidobacterium animalis* DN-173 010 strains were similar for dextrin D2 and glucose fermentation (Table 1). The concentration of acetic acid resulting from the fermentation of dextrin D2 was much lower than that obtained from glucose fermentation, but the figures for dextrin D1 and glucose fermentation were similar. The other acids (propionic and formic), as well as acetaldehyde and ethanol, were produced in similar quantities in the presence of both resistant dextrins and glucose (Table 1).

PRODUCTS OF RESISTANT DEXTRIN FERMENTATION GENERATED BY BACTERIA ISOLATED FROM FECES

All bacteria isolated from human feces produced lactic, acetic, and succinic acids. Furthermore, formic acid was generated by *Escherichia coli* and *Enterococcus* strains, butyric acid by *Clostridium* strains, and propionic acid by *Bacteroides* strains, all irrespective of dextrin type and volunteers' age. All strains produced small quantities of acetaldehyde and, except for *Bacteroides*, slight amounts of ethanol (Table 2).

The main product of fermentation of resistant dextrins was lactic acid. Its concentration depended on the type of bacteria and age of the person from whom they were isolated. The highest amount of lactic acid was produced by the strains isolated from the feces of adults (54 to 116 mg 100 mL^{-1}), and the smallest by those isolated from 1-year-old children (31 to 70 mg 100 mL^{-1}). Among the studied bacterial isolates, the highest quantities of lactic acid were generated by *Escherichia coli* and *Enterococcus*; *Bacteroides* produced approximately 50% less of this acid (Table 2).

The concentration of acetic acid varied depending on the age of the person from whom the bacteria were isolated and ranged from 4 to 76 mg 100 mL^{-1} (Table 2). Irrespective of the type of resistant dextrin, acetic acid was produced at the highest rate by the strains isolated from the feces of 30-year-olds – 40% to 60% more than those isolated from the feces of 1-year-olds (Table 2). The highest quantities of this acid were produced as a result of resistant dextrins by *Clostridium* strains (53 to 76 mg mL^{-1}), and the lowest by *Enterococcus* (3 to 7 mg 100 mL^{-1}) (Table 2).

All the strains produced succinic acid in media with resistant dextrins, but the concentration of this metabolite was low and ranged from 1 to 30 mg 100 mL^{-1}. *Escherichia coli* and *Clostridium* strains were similar in respect of the

Table 1. Concentration of fermentation products after 24-h cultivation of probiotic bacteria in medium containing resistant dextrins as the only source of carbon

Bacteria	Carbon source	Fermentation products mg 100 mL^{-1}	SD	L:A:P:F
Lactobacillus casei Shirota	D1	69.05	0.12	77:20:3:0
	D2	115.45	0.11	95:4:1:0
	Glucose	129.30	0.08	79:17:4:0
Lactobacillus casei DN 114 001	D1	90.10	0.09	82:16:2:0
	D2	120.67	0.12	95:4:1:0
	Glucose	128.78	0.10	79:17:4:0
Lactobacillus rhamnosus Lakcid	D1	98.45	0.05	81:16:3:0
	D2	113.95	0.05	96:3:0:0
	Glucose	325.11	0.12	93:6:1:0
Bifidobacterium animalis DN 173 010	D1	165.56	0.09	49:30:1:20
	D2	183.60	0.11	61:10:2:27
	Glucose	193.78	0.05	54:22:2:22
Bifidobacterium bifidum Bb12	D1	157.05	0.01	47:30:2:21
	D2	178.75	0.012	60:10:2:28
	Glucose	202.75	0.011	51:26:2:21

L – lactic acid; A – acetic acid; P – propionic acid; F – formic acid; SD standard deviation

amount of succinic acid they generated; *Bacteroides* strains produced the highest quantity of this acid (8 to 29.4 mg 100 mL^{-1}), while *Enterococcus* strains as much as 15 times less, irrespective of the type of resistant dextrin (Table 2). *Escherichia coli* and *Enterococcus* strains produced similar quantities of formic acid, from 60 to 92 mg 100 mL^{-1}. The concentration of this acid was not found to vary with the age of the persons from whom the strains were isolated (Table 2).

Bacteroides strains produced a considerable quantity of propionic acid (200 to 494 mg 100 mL^{1}), which accounted for approximately 79% to 86% of the overall amount of all the acids produced (Table 2). The bacteria isolated from the feces of adults and 8-year-olds were similar in terms of how much propionic acid they produced, while strains isolated from the feces of 1-year-olds generated 50% less of this acid. The fermentation of resistant dextrins yielded butyric acid only in *Clostridium* isolates (54 to 82 mg 100 mL^{-1}) (Table 2). All strains generated small amounts of acetaldehyde (0.1 to 6 mg 100 mL^{-1}) and ethanol (0.02 to 0.09 mg mL^{-1}) (Table 2).

In media containing dextrins (D1, D2), all the studied strains produced slightly lower amounts of fermentation products than in the case of fermentation of glucose (Table 2).

Over the past several years, researchers have emphasized that short-chain fatty acids (SCFAs) lower the pH of intestinal contents and stimulate the growth of intestinal epithelium (butyric acid), hepatocytes (propionic acid), and peripheral tissues (acetic acids). SCFAs also have an effect on the mineral balance in the human body by stimulating the absorption of calcium, magnesium, and iron ions from the large intestine. The products formed in the process of fermentation are used by the cells of the mucous membrane and serve as a source of both carbon and energy for microorganisms [4,13,29,37]. The mechanism of formation of fermentation products from the compounds passed to the final segment of the gastrointestinal tract depends on the strains that carry out the fermentation process, their enzymatic capabilities, and the substrate subjected to fermentation. One of the requirements that prebiotics are supposed to meet is that they should not be digested in the upper parts of the digestive system, and thus be transferred to the large intestine in an unchanged state to serve as a substrate for fermentation by the microbiota of the large intestine. The type of metabolites formed depends on the type of fermentation conducted by the microorganisms.

During the fermentation of resistant dextrins (potential prebiotics), probiotic lactobacilli predominantly produced lactic and acetic acids. *Bifidobacterium* strains additionally generated formic acid. The intestinal bacteria *Escherichia coli* and *Enterococcus* mostly produced lactic, formic, and acetic acids; *Clostridium* strains produced primarily butyric acid; and *Bacteroides* strains produced primarily propionic acid. This means that these bacteria metabolized resistant dextrins in the typical process of lactic, formic, propionic, or butyric fermentation, or through mixed fermentation. The manner of production of resistant dextrins did not significantly influence the type and concentration of fermentation products.

GROWTH OF PROBIOTIC AND INTESTINAL BACTERIA IN THE PRESENCE OF RESISTANT DEXTRINS (CO-CULTURES)

In the stationary phase, that is, after 24 h of incubation, the cultures were dominated by probiotic *Lactobacillus* and *Bifidobacterium* strains, jointly accounting for over 34% of the microbial population. The increase in the number of these bacteria (N_k–N_o) at 24 h of incubation was in the range 1.15–1.44 log CFU mL^{-1} and 0.99–1.16 log CFU mL^{-1}, respectively, depending on the type of dextrin used. The increase in the number of probiotic bacteria (Nk–No) at 24 h of incubation was lower in the control medium (0.75–0.98 log CFU mL^{-1} and 0.45–0.98 log CFU mL^{-1}) (Table 3, 4, 5). This demonstrates the ability of probiotic bacteria to use dextrins. Also *Escherichia coli* strains were observed to grow well. They accounted for 17% of the overall population, except for isolates from 30-year-olds (Table 3, 4). The growth of *Bacteroides* and *Enterococcus* strains was similar and irrespective of host age. *Clostridium* strains, which accounted for approximately 16% of the overall population, were found to grow at the slowest rate (Table 3, 4). At the end of incubation, that is, after 168 h, probiotic lactobacilli and bifidobacteria exhibited high viability. Their cell counts were by one or two orders of magnitude higher than those of *Escherichia coli*, *Enterococcus*, *Clostridium*, and *Bacteroides* intestinal isolates. After 168 h of incubation, probiotic bacteria accounted for 46% of the overall bacterial population, intestinal *Clostridium* strains for 9%–14%, while *Enterococcus* and *Escherichia coli* strains accounted for 13-18%, depending on host age and dextrin type.

In media without saccharides (control cultures), *Enterococcus*, *Clostridium*, and *Bacteroides* strains isolated from the feces of 1-year-olds and *Clostridium* strains isolated from the feces of 8-year-olds were not found to grow at all (Table 5). The increase in the number of other bacteria was small, with the cell counts ranging from 0.28 to 0.94 CFU mL^{-1}. At the end of the culture, the number of probiotic and intestinal bacteria in media with dextrins was higher by 3 to 4 orders of magnitude than in media without saccharides.

No significant differences were observed between the growth of bacteria isolated from the feces of 1-year-olds and that of bacteria acquired from 8-year-olds. In contrast, the growth rate of the strains isolated from the feces of 30-year-olds was much smaller in media with dextrin D1, which may indicate greater selectivity of this dextrin in respect of *Clostridium*, *Bacteroides*, and *Enterococcus* strains isolated from people at different ages.

Co-cultures were dominated by probiotic *Bifidobacterium* and *Lactobacillus* strains, irrespective of dextrin type and

Table 2. Fermentation products after 24-h cultivation of intestinal bacteria in broth containing resistant dextrins as the only source of carbon

Children 1 – years				
Bacteria	**Carbon source**	**Fermentation products mg 100 mL^{-1}**	**SD**	**L:A:P:S:F:B**
Escherichia coli	D1	166.12	0.10	43:7:0:2:48:0
	D2	162.62	0.02	41:6:0:3:50:0
	Glucose	170.03	0.09	42:7:0:3:48:0
Enterococcus	D1	131.76	0.05	50:2:0:1:47:0
	D2	130.56	0.10	50:2:0:1:47:0
	Glucose	138.86	0.05	52:2:0:1:45:0
Clostridium	D1	206.66	0.03	25:31:0:4:0:40
	D2	195.66	0.08	26:29:0:4:0:41
	Glucose	214.27	0.01	25:32:0:3:0:40
Bacteroides	D1	261.50	0.04	12:2:79:7:0:0
	D2	246.00	0.03	13:2:82:3:0:0
	Glucose	308.20	0.04	15:3:79:3:0:0
Children 8 – years				
Escherichia coli	D1	195.24	0.10	44:9:0:3:44:0
	D2	200.94	0.01	41:9:0:3:47:0
	Glucose	198.14	0.05	43:10:0:3:44:0
Enterococcus	D1	151.27	0.06	55:3:0:1:42:0
	D2	151.37	0.18	55:3:0:1:41:0
	Glucose	161.39	0.10	56:3:0:1:40:0
Clostridium	D1	146.26	0.06	20:38:0:4:0:38
	D2	149.87	0.08	20:40:0:3:0:37
	Glucose	142.66	0.02	21:37:0:4:0:38
Bacteroides	D1	477.40	0.01	10:2:86:2:0:0
	D2	470.40	0.03	10:2:86:2:0:0
	Glucose	526.40	0.07	12:2:83:3:0:0
Adult 30– years				
Escherichia coli	D1	224.15	0.02	48:9:0:3:40:0
	D2	235.36	0.10	50:8:0:3:39:0
	Glucose	240.56	0.05	49:8:0:3:40:0
Enterococcus	D1	194.29	0.05	63:4:0:1:32:0
	D2	190.49	0.03	63:3:0:1:33:0
	Glucose	238.30	0.08	69:3:0:1:27:0
Clostridium	D1	236.47	0.01	1:31:0:4:0:34
	D2	237.27	0.06	31:30:0:4:0:35
	Glucose	261.87	0.02	29:34:0:3:0:34
Bacteroides	D1	602.50	0.04	10:2:83:5:0:0
	D2	595.30	0.08	9:2:84:5:0:0
	Glucose	688.50	0.02	10:3:81:6:0:0

L – lactic acid; A – acetic acid; P – propionic acid; F – formic acid. SD standard deviation

host age. However, intestinal strains were also capable of growth, with *Escherichia coli* growing quite intensively. Thus, a diet including prebiotics may enhance the development of *E. coli* strains [3,32]. Still, it should be noted that they are deemed to have beneficial effects on the human gastrointestinal system [12,23]. In co-cultures of probiotic and intestinal bacteria in media with resistant dextrins, the bacterial counts were slightly higher (2% to 5%) than in pure cultures of those bacteria. It is thought that the higher numbers of probiotic bacteria and bacteria isolated from human feces may have been caused by the interactions occurring in a mixture, including multistep protocooperation or metabiosis.

The growth of probiotic lactobacilli and bifidobacteria, as well as *Escherichia coli*, *Enterococcus*, *Bacteroides*, and *Clostridium* strains isolated from human feces, shows

that both studied dextrins (D1 and D2) were utilized as a source of assimilable carbon and energy by the strains. Furthermore, the higher counts of probiotic bacteria than those of fecal isolates indicate that the studied resistant dextrins have a selective effect.

PREBIOTIC INDEX

The values of the prebiotic index (PI) in media with dextrins were positive and increased with time of culture, which shows that probiotic *Bifidobacterium* and *Lactobacillus* strains were capable of dominating their environment in mixtures with intestinal bacteria cultured in media containing resistant dextrins. The lowest PI values were found for the co-culture of probiotic bacteria and those isolated from the feces of 1-year-olds, and the highest for the co-culture containing bacteria acqu-

Table 3. Increase in number (N_k–N_o) log CFU mL^{-1} of probiotic *Bifidobacterium* and *Lactobacillus* in co-culture with bacteria isolated from feces of children aged 1 year or 8 years and adults aged 30 years. 24 h of cultivation in medium with dextrin D1

Age volunteers	(N_k − N_0) log CFU mL^{-1} + - SD					
	Lactobacillus	*Bifidobacterium*	*Escherichia*	*Enterococcus*	*Clostridium*	*Bacteroides*
1	1.44 $^+_-$ 0.03	1.04 $^+_-$ 0.10	1.05 $^+_-$ 0.07	0.79 $^+_-$ 0.01	1.00 $^+_-$ 0.02	0.54 $^+_-$ 0.08
8	1.34 $^+_-$ 0.10	1.08 $^+_-$ 0.02	1.19 $^+_-$ 0.03	1.04 $^+_-$ 0.10	0.78 $^+_-$ 0.10	0.44 $^+_-$ 0.09
30	1.22 $^+_-$ 0.20	0.99 $^+_-$ 0.08	1.12 $^+_-$ 0.05	0.95 $^+_-$ 0.06	0.56 $^+_-$ 0.03	0.49 $^+_-$ 0.01

SD standard deviation
N_K – the number of bacterial cells at 24 h of incubation
N_0 – the number of bacterial cells at inoculation

Table 4. Increase in number (N_k–N_o) log CFU mL^{-1} of probiotic *Bifidobacterium* and *Lactobacillus* in co-culture with bacteria isolated from feces of children aged 1 year or 8 years and adults aged 30 years. 24 h of cultivation in medium with dextrin D2

Age volunteers	(N_k − N_0) log CFU mL^{-1} + - SD					
	Lactobacillus	*Bifidobacterium*	*Escherichia*	*Enterococcus*	*Clostridium*	*Bacteroides*
1	1.37 $^+_-$ 0.20	1.08 $^+_-$ 0.03	1.05 $^+_-$ 0.07	0.81 $^+_-$ 0.04	0.83 $^+_-$ 0.03	0.40 $^+_-$ 0.11
8	1.15 $^+_-$ 0.01	1.16 $^+_-$ 0.10	1.14 $^+_-$ 0.06	0.88 $^+_-$ 0.15	0.73 $^+_-$ 0.11	0.71 $^+_-$ 0.04
30	1.18 $^+_-$ 0.02	1.01 $^+_-$ 0.09	0.20 $^+_-$ 0.08	0.62 $^+_-$ 0.12	1.12 $^+_-$ 0.05	0.52 $^+_-$ 0.01

SD standard deviation
N_K – the number of bacterial cells at 24 h of incubation
N_0 – the number of bacterial cells at inoculation

Table 5. Increase in number (N_k–N_o) log CFU mL^{-1} of probiotic *Bifidobacterium* and *Lactobacillus* in co-culture with bacteria isolated from feces of children aged 1 year or 8 years and adults aged 30 years. 24 h of cultivation in medium control

Age volunteers	(N_k − N_0) log CFU mL^{-1} + - SD					
	Lactobacillus	*Bifidobacterium*	*Escherichia*	*Enterococcus*	*Clostridium*	*Bacteroides*
1	0.98 $^+_-$ 0.11	0.88 $^+_-$ 0.03	0.63 $^+_-$ 0.01	-0.04 $^+_-$ 0.01	-0.32 $^+_-$ 0.03	-0.32 $^+_-$ 0.01
8	0.75 $^+_-$ 0.06	0.80 $^+_-$ 0.01	0.64 $^+_-$ 0.02	0.72 $^+_-$ 0.15	0.28 $^+_-$ 0.01	-0.18 $^+_-$ 0.02
30	0.87 $^+_-$ 0.09	0.45 $^+_-$ 0.03	0.94 $^+_-$ 0.01	0.49 $^+_-$ 0.05	0.85 $^+_-$ 0.05	0.36 $^+_-$ 0.01

SD standard deviation
N_K – the number of bacterial cells at 24 h of incubation
N_0 – the number of bacterial cells at inoculation

ired from 8-year-olds. Furthermore, PI values were higher in cultures containing dextrin D1 than D2 (Table 6).

Table 6. Prebiotic index values for co-cultures of probiotic *Bifidobacterium* and *Lactobacillus* strains and intestinal *Clostridium* and *Bacteroides* strains in media with resistant dextrins D1 and D2

Dextrin	Age volunteers	Incubation time (h)				
		24	48	72	96	168
D1	1	0.101	0.192	0.222	0.316	0.438
	8	0.133	0.144	0.394	0.878	1.069
	30	0.109	0.180	0.182	0.457	0.698
D2	1	0.141	0.161	0.140	0.198	0.599
	8	0.068	0.164	0.268	0.490	0.714
	30	0.038	0.030	0.204	0.272	0.877

This shows that prebiotic index values should be considered individually for different host age groups due to the specific microbiota of the gastrointestinal tract and the prevailing bacterial species, which change with host age [34]. The prebiotic index values for both resistant dextrins were higher than those determined by Olano-Martin et al. (2002) [32] for oligosaccharides (POS I, POS II) under the same incubation conditions. Of the two studied dextrins, D1, which was produced in the presence of citric acid, exhibited a higher prebiotic index than D2, which was produced in the presence of tartaric acid. This proves that dextrin D1 more strongly stimulates the growth of *Bifidobacterium* and *Lactobacillus* strains.

Conclusion

Dextrin obtained in the presence of citric acid and heated for four hours (D1) and dextrin obtained in the presence of tartaric acid and heated for two hours (D2) may be considered substances with prebiotic properties due to their chemical and physical properties and selectivity towards the studied probiotic bacterial strains. According to the guidelines of FAO experts concerning the use of prebiotics, it seems necessary to conduct more randomized and controlled studies with appropriate statistical power in this field [11]. The prebiotic properties of dextrins D1 and D2 are currently being investigated in further *in vivo* studies.

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