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# Lactose-free milk enriched with resistant dextrin

## Mleko bez laktozy wzbogacone oporną dekstryną

**Renata Barczynska** [A B C D E], **Iwona Zawierucha** [B E], **Katarzyna Bandurska** [F], **Janusz Kapusniak** [G]

Institute of Chemistry, Environmental Protection and Biotechnology, Jan Dlugosz University in Czestochowa, Poland

## Summary

**Aim:** The aim of the study was to check whether the resistant dextrin obtained from potato starch, as a substance with prebiotic properties, activates the growth and development of selected intestinal bacteria strains in a ready food product – lactose-free milk.

**Material/Methods:** The research involved the use of dextrin from potato starch obtained in accordance with the patent no. PL. 220965. This dextrin and strains of bacteria *Lactobacillus, Bifidobacterium, Bacteroides, Clostridium, Prevotella* were added to lactose-free milk. The growth of bacteria, type and concentration of short-chain fatty acids and branched fatty acids were checked for the tested milk and the prebiotic index was determined.

**Results:** After 48 hours in lactose-free milk with the addition of dextrin, the dominance of *Lactobacillus* and *Bifidobacterium* over *Clostridium* and *Bacteroides* strains was demonstrated. The number of lactic acid bacteria beneficial to health was 35% higher than the numbers of *Clostridium* and *Bacteroides*. The prebiotic index determined after 24h was 0.191, and after 48h it increased to 0.213. Supplementation of lactose-free milk with resistant dextrin contributed to the increase in lactic acid by 57%, total concentration of SCFA by 23%, and in the reduction of the negative putrefactive SCFA concentration by 49% in relation to control sample milk without lactose and no dextrin.

**Conclusions:** Lactose-free milk supplemented with dextrin may favorably affect the intestinal microbiota system of people with lactose intolerance, reduce digestive processes in the intestine.

**Keywords:** **potato dextrin • lactose - free milk • lactose intolerance • short-chain fatty acids**

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**Author's address:** Renata Barczynska, Institute of Chemistry, Environmental Protection and Biotechnology, Jan Dlugosz University in Czestochowa, Armii Krajowej 13/15, 42-200 Czestochowa, Poland; e-mail: r.barczynska@gmail.com

**Abbreviations:** **SCFA** – short-chain fatty acids, **BCFA** – branched fatty acids, **HPLC** - High Performance Liquid Chromatography, **PI** – prebiotic index, **MWL** - the lactose-free milk, control sample, **MWL + D** – the lactose-free milk with the addition of dextrin.

## INTRODUCTION

In recent years, a dynamic development of the functional food market has been observed, along with an increase in consumer attention to the quality of consumed food. Products enriched with substances that improve their properties, such as dietary fiber and prebiotics, are becoming increasingly popular.

Lactose intolerance, depending on the geographical region, may range from several to several dozen percent of the population of a given country. The reason for lactose intolerance is the lack or impairment in lactase enzyme activity. Several types of lactase deficiency have been distinguished such as the following: inborn, primary and acquired lactase deficiency [24, 27]. Undigested lactose is an osmotic charge that causes the fluid to travel to the gastrointestinal tract. In the large intestine lactose undergoes bacterial fermentation, resulting in the formation of preferred SCFA as well as water and gases, such as carbon dioxide and hydrogen. Methane, hydrogen sulphide and acetates may also form as a result of further bacterial transformation of hydrogen. In the case of lactose intolerance, the consequence of poor digestion and absorption of lactose are diarrhea, flatulence, abdominal pain, excessive gas rejection as well as adverse changes in intestinal microbiota [15, 24, 27]. Lactose is the dominant saccharide of milk, made of glucose and galactose. It is found in many food products, especially in milk and its dairy products. The content of lactose in these products ranges from 4.4% to 5.2% [12, 18]. People with lactose intolerance should not give up dairy products, because the dairy industry offers a wide range of lactose-free milk products. However, we observe that grocery products offered in the market have low availability of lactose-free products enriched with prebiotic.

Gibson et al. (2017) define a prebiotic as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" [14]. Prebiotics are not hydrolyzed or absorbed in the upper parts of the gastrointestinal tract, due to which they enter the colon in unaltered form, where they serve as nutrients to beneficial bacteria [25]. The most important function of prebiotics in the human body is modulation of the colonic microbiota by selective stimulation of the growth and activity of some strains. Moreover, research efforts are underway to elucidate the anti-pathogenic and anticancer effects of prebiotics, as well as their preventive activity against colonic diseases linked to inhibited adhesion of pathogenic microbes to the intestinal mucosa [9, 17, 19].

A new substance with prebiotic properties is resistant dextrin from potato starch. It was also subjected to simultaneous thermolysis and chemical modification in the presence of volatile inorganic acid (hydrochloric acid) as a catalyst in the dextrinization process, and organic acid (citric acid) as a modifying agent [3, 16]. It was shown that the solubility of dextrin was established at 63%. It was demonstrated that the average molecular weight (Mw) of dextrin obtained with the use of citric acid was estimated at $4.8\times10^3$ g/mol (average DP 25 – 30) [3, 16].

However, from the viewpoint of resistance to amylolytic enzymes in the gastrointestinal tract, not only the molecular weight of the product is important, but also its chemical structure. The number and type of branching occurring in the molecules seem to be very important. Studies involving high performance anion exchange chromatography (HPAEC) showed that the average chain length of dextrins was lower than the average DP of the main fraction, which denoted the occurrence of branches in dextrin molecules [3, 16]. Dextrinisation of starch in the presence of citric acid led to an increase in the undigested fraction, up to 70%. Dextrin obtained from potato starch met the basic condition for prebiotics; they were not subjected to decomposition by digestive enzymes in initial sections of the digestive tract [3].

The main objective of the study was to check whether the resistant dextrin obtained from potato starch as a substance with prebiotic properties activates the growth and development of selected intestinal bacteria strains in the finished food product – lactose-free milk, as well as to develop a new food product: lactose-free milk enriched with prebiotic formula.

## MATERIALS AND METHOD

### Dextrin

The dextrin used in the study was produced at the Department of Biochemistry and Technology of Bioproducts, Jan Dlugosz University in Czestochowa. The dextrin was obtained according to patent no. PL. 220965 [5, 6]. Thus, potato starch was sprayed with a hydrochloric acid solution (0.5% w/w) to obtain a final HCl concentration of 0.1% on a dry starch basis (dsb). A citric acid solution (0.5% w/v) was then added to obtain a final organic acid concentration of 0.1% dsb. The thoroughly mixed sample was dried at $1100^0$C to obtain a final moisture content below 5%. The dried sample (10 g) was placed in an anti-pressure bottle (SIMAX), capped, and heated at $1300^0$C for 3 hours in an Economy Laboratory Furnace (ELF) model 11/6 (Carbolite, Hope, England). Products were cooled in a desiccator and milled into powder with a particle size of <1 mm. Dietary fiber preparations were then washed with ethanol (80%, v/v) to remove excess tartaric acid and low molecular weight material formed during dextrinization, dried overnight at $500^0$C, dried at $1100^0$C for 1 hour following this period, and finally milled in a cyclone lab sample mill (UDY Corp., Fort Collins, CO, USA) fitted with a 0.50 mm screen.

### Milk

The UHT lactose-free milk from a Polish producer, which possesses a reduced carbohydrate content, was tested. The lactose content guaranteed by the producer was <0.01 g 100 $mL^{-1}$; fat 1.5g 100$mL^{-1}$; carbohydrates 4.7g 100 $mL^{-1}$, protein 3.0g 100$mL^{-1}$, calcium 105mg 100$mL^{-1}$. The milk was stored according to the manufacturer's instructions.

### Bacteria

Strains of *Lactobacillus*, *Bifidobacterium*, *Bacteroides*, *Clostridium*, and *Prevotella* were used in the studies. These bacteria came from the own collection, previously isolated from the faeces of 10 healthy children. For these bacteria, genomic DNA was isolated, amplified using PCR and genetic identification based on sequencing of the 16S rRNA gene was made.

## BACTERIA CULTURE

Prior to experiments bacteria were activated by two-fold inoculation (3%): *Lactobacillus*, *Bifidobacterium*, Rogosa and Sharpe (MRS) broth, *Bacteroides* and *Clostridium* in liquid Viande Levure (VL) broth, and *Prevotella* on Brucella bullion. Next, the bacterial inoculum was prepared until the number of individual bacteria reached $10^7$-$10^8$ cfu $mL^{-1}$ (colony forming units $mL^{-1}$), which corresponds to the number of cells of these microorganisms in the initial section of the colon. The prepared inoculum was inoculated into 250 ml of lactose-free milk to which 5% of resistant dextrin was added. The control sample consisted of lactose-free milk inoculated with the same inoculum as tested but without the addition of resistant dextrin. Incubation of milk was carried out for 48 hours at $37^0$C maintaining anaerobic conditions. After 24 hours of incubation and at the end of the experiment, which was after 48 hours, the bacteria number was determined using the classical breeding method, inoculating *Lactobacillus* bacteria on ROGOSA medium, *Bifidobacterium* on RCA medium, *Bacteroides* on Schaedler, *Clostridium* on DRCM, *Prevotella* on Brucella. The plates were incubated at $37^0$C for 48 hours while preserving aerobic conditions for *Lactobacillus*, and anaerobic conditions for *Bifidobacterium*, *Bacteroides*, *Clostridium* and *Prevotella*. The results were given in cfu $mL^{-1}$. The experiment was repeated three times.

## DETERMINATION OF FERMENTATION PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Determination of lactic acid, SCFA (acetic, propionic, butyric, formic, and valeric) and branched fatty acids (BCFA) as isovaleric and isobutyric were obtained by high-performance liquid chromatography (HPLC) using

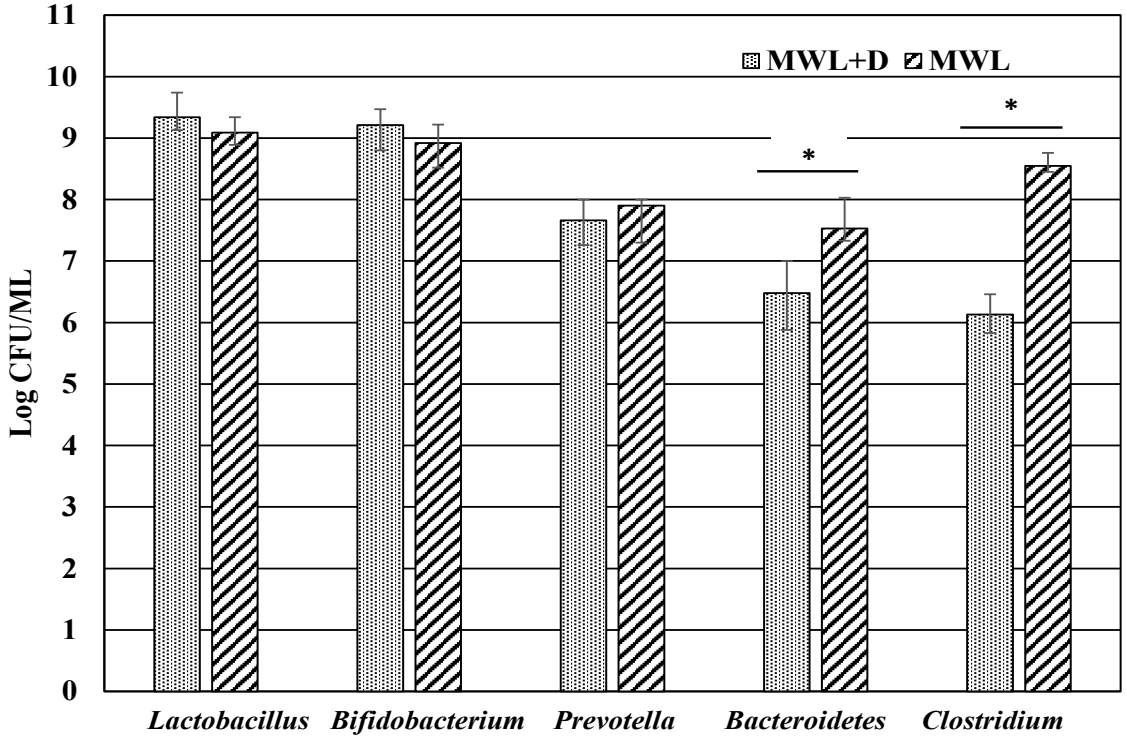


**Fig. 1.** Numbers of bacteria after 24 hours of the experiment in the lactose-free milk with the addition of dextrin (MWL+D) and lactose-free milk (MWL) control sample

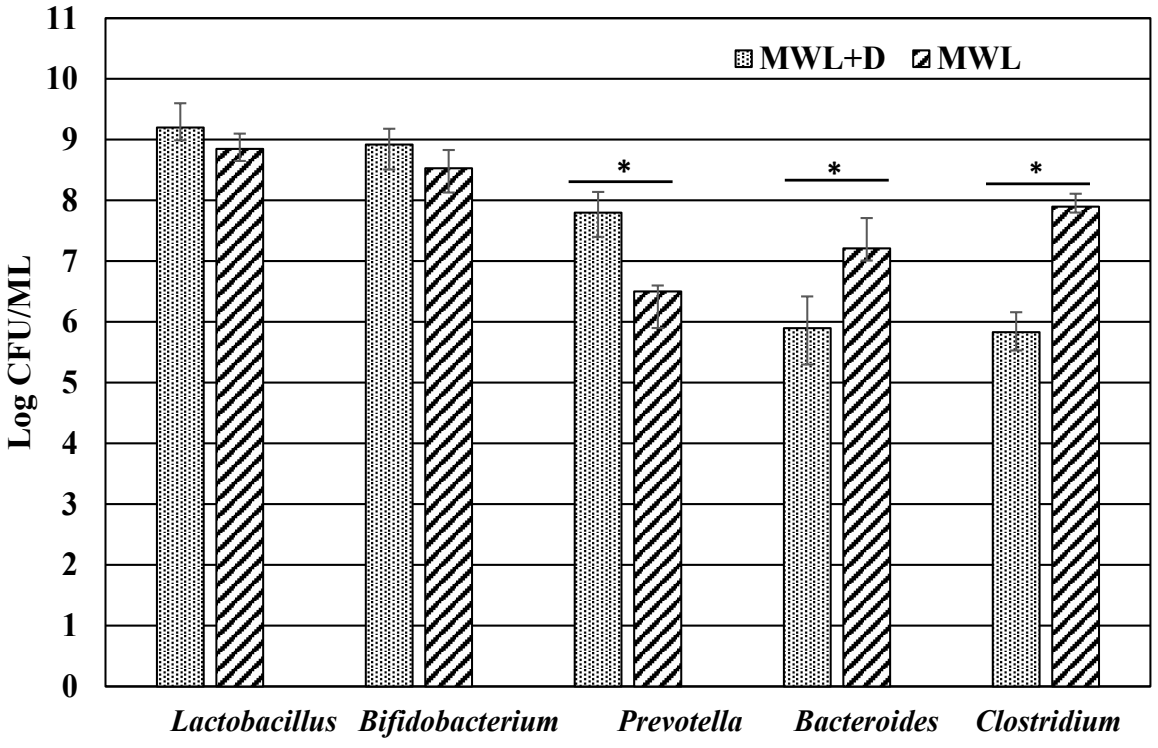


**Fig. 2**. Numbers of bacteria after 48 hours of the experiment in the lactose-free milk with the addition of dextrin (MWL+D) and lactose-free milk (MWL) control sample

a Surveyor chromatograph (Thermo Scientific) and an Aminex HPX-87H column (300 × 7.8 mm) from Bio-Rad Aminex® with sulfonated divinyl benzene-styrene copolymer support. The following analytical parameters were used: 300 × 7.8 mm Aminex HPX-87H column, mobile phase 0.005 M H2SO4, 210 nm UV detector, injector valve with a sample loop, injection volume 10 µL, column temperature 60°C, flow rate 0.6 µL $min^{-1}$, analysis of a single sample 35 min. Samples with known concentrations of the acids (0; 0.125; 0.25; 0.50; 0.75 and 1 % acid $mL^{-1}$) were analyzed with HPLC in order to obtain calibration curves showing acid concentration to peak area ratios.

## DETERMINATION OF PREBIOTIC INDEX (PI)

Prebiotic index (PI) was analyzed using quantitative equation [22]

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

where PI is prebiotic index;

Bif, number of bifidobacterium cells at sample time/numbers at inoculation;

Bac, number of bacteroides cells at sample time/numbers at inoculation;

Lac, number of lactobacilli cells at sample time/numbers at inoculation;

Clos, number of clostridia cells at sample time/numbers at inoculation;

Total number of bacteria cells at sample time/numbers at inoculation. The sum of bacteria *Bacteroides, Clostridium, Lactobacillus, Bifidobacterium*.

## STATISTICAL ANALYSIS

The results were evaluated with the W-Shapiro Wilk test assessing the normality of the distribution of the results. Due to the deviation from the normal distribution, further analysis was based on the U Mann-Whitney test. Statistical significance was established at $p<0.05$. The statistical analysis was performed using the STATISTICA 10.0 software (StatSoft, Inc.).

## RESULTS

After 24 hours of the experiment in the lactose-free milk with the addition of dextrin (MWL +D), the sample exhibited the highest number of bacteria belonging to *Lactobacillus* and *Bifidobacterium* (9.34 and 9.31 log cfu $mL^{-1}$) strains. There was no statistically significant difference in the growth of these strains compared to the lactose-free milk

**Table 1**. Prebiotic index values for lactose-free milk with the addition of resistant dextrin from potato starch

| Prebiotics index | Incubation time (h) | |
|---|---|---|
| | 24 | 48 |
| | 0.191* | 0.213* |

* - mean value of three measurements

(MWL) control sample. The number of *Clostridium* and *Bacteroides* strains was significantly lower ($p < 0.05$) in MWL + D sample (6.48 and 6.13log cfu $mL^{-1}$) than in MWL sample (7.53 and 8.55log cfu $mL^{-1}$) (Fig.1). After 48 hours of incubation of the MWL + D sample, the dominance of strains of the genus *Lactobacillus* and *Bifidobacterium* over *Clostridium* and *Bacteroides* was demonstrated. The number of lactic acid bacteria beneficial for health was for *Lactobacillus* 9.2 log cfu $mL^{-1}$and for *Bifidobacterium* 8.92 log cfu $mL^{-1}$, while the numbers of *Clostridium* and *Bacteroides* strains were few orders of magnitude smaller 5.83 and 5.9 log cfu $mL^{-1}$, respectively. When the number of examined bacterial strains in the MWL + D and MWL test samples were compared after 48 hours of incubation, statistically significant differences in the growth of *Clostridium, Bacteroides* and *Prevotella* strains were found. The number of *Clostridium* and *Bacteroides* strains were lower in lactose-free milk enriched with dextrins than in lactose-free milk without dextrin ($p < 0.05$), while for *Prevotella* strains, the reverse results were found; the number of these strains was higher in the sample with dextrin 7.8 log cfu $mL^{-1}$ than in the sample with no dextrin 6.5 log cfu $mL^{-1}$ ($p < 0.05$) (Fig. 2). After 24 and 48 hours of incubation of MWL + D and MWL tests samples, a lower pH was found for the MWL + D sample (after 24h 4.8, after 48h 4.2) than in the MWL sample (after 24h 5.6, after 48h 5.2 respectively).

The prebiotic index for lactose-free milk with the addition of dextrin was positive and increased with the incubation time. After 24 hours of incubation it was 0.091, while after 48 hours of incubation it was 0.213 (Table 1).

After the experiment, i.e. after 48 hours of incubation, the lactic acid concentration was more than twice higher in the MWL +D (490 mg $100mL^{-1}$) test sample than in the MWL test sample (210 mg $100mL^{-1}$) ($p < 0.001$). The total concentration of SCFA was also higher in lactose-free milk with the addition of dextrin (725 mg $100mL^{-1}$) than in lactose-free milk without dextrin (561 mg $100mL^{-1}$). Of all SCFA (acetic, propionic, formic, butyric, valeric) the highest concentration was found for acetic acid in both MWL + D sample (340 mg $100mL^{-1}$) and in MWL sample (270 mg $100mL^{-1}$), the lowest concentration was noted for valeric acid in MWL + D sample (43 mg $100mL^{-1}$) and butyric acid in MWL sample (35 mg $100mL^{-1}$). The concentrations of the remaining SCFAs ranged from 92up to 129 mg $100mL^{-1}$ for MWL + D and from 58up to 101 mg $100mL^{-1}$for MWL. The total concentration of branched chain fatty acids (BCFA) was significantly lower in lactose-free milk with the addition of dextrin 24 mg/100ml than in lactose-free milk without dextrin 73 mg $100mL^{-1}$ ($p < 0.001$) (Table 2).

## DISCUSSION

The dextrin used in the studies in terms of structure and properties is resistant to enzymatic digestion obtained through dextrination of potato starch acidified with hydrochloric and citric acids in strictly controlled conditions [8]. In the earlier studies by Barczynska et al., 2010, 2012; Jochym et al., 2012 [3, 4, 7, 16] the prebiotic properties of the used dextrin were showed. It was a source of carbon for intestinal strains and additionally stimulated the growth of strains belonging to the genus of *Lactobacillus* and *Bifidobacterium* with both proven probiotic properties and isolated from the faeces of people of different ages and limited the growth of strains belonging to the genus of *Clostridium* and *Bacteroidetes* [3, 4, 16]. Bearing in mind the prebiotic properties of resistant dextrin obtained from potato starch, we started research on the possibility of using dextrin in food products, for example on the possibility of enriching lactose-free milk with dextrin. Lactose-free milk is intended for people who, for various reasons, do not have a lactase enzyme in their body or have an enzyme but its activity is damaged in various degrees [24, 27]. Consuming lactose-free milk enriched with prebiotic, i.e. resistant dextrin from potato starch, could contribute to many beneficial effects such as the increase in the activity of the beneficial intestinal strains of *Lactobacillus*, *Bifidobacterium*, lowering the intestinal pH, increasing the concentration of short-chain fatty acids and increasing the absorption of mineral compounds, especially calcium from milk [1, 10, 19, 26].

In presented studies, it was shown that the addition of dextrin into lactose-free milk selectively stimulated the growth of specific groups of bacteria. After 24 hours of incubation, the dominant strains were *Lactobacillus* and *Bifidobacterium*, while the number of the remaining tested strains was lower (*Clostridium, Bacteroides* and *Prevotella*). Extending the incubation time up to 48 hours resulted in even more favorable ratios of *Lactobacillus* and *Bifidobacterium* to *Clostridium* and *Bacteroides*. Strains of *Lactobacillus* and *Bifidobacterium* grew in the control sample containing lactose-free milk without dextrin as well as in lactose-free milk with addition of dextrin. However, other strains such as *Clostridium*, *Prevotella* and *Bacteroides* grew equally well, and therefore in the control sample no selectivity was observed in relation to unfavorable grafts.

In earlier studies, Barczynska et al. [3, 4, 16] demonstrated the selective stimulation of the growth of beneficial *Lactobacillus* and *Bifidobacterium* strains; however, these studies were performed in culture media. Therefore,

**Table 2**. Concentration of SCFA and BCFA in lactose-free milk with the addition of resistant dextrin from potato starch (MWL+D) and in lactose-free milk (MWL)

| | MWL+D | | MWL | | p |
|---|---|---|---|---|---|
| | Acid concentration [mg100 mL$^{-1}$] | Average [mg100 mL$^{-1}$] | Acid concentration [mg100 mL$^{-1}$] | Average [mg100 mL$^{-1}$] | |
| **Lactic acid** | 419-577 | 490 | 177-220 | 210 | < 0.001 |
| **SCFA** | | | | | |
| **Acetic** | 338-351 | 340 | 218-282 | 270 | < 0.001 |
| **Propionic** | 95-133 | 129 | 92-127 | 101 | < 0.05 |
| **Butyric** | 91-118 | 92 | 22-55 | 35 | < 0.001 |
| **Formic** | 93-128 | 121 | 99-114 | 97 | < 0.05 |
| **Valeric** | 32-55 | 43 | 42-65 | 58 | < 0.05 |
| **Total** | 649-85 | 725 | 473-643 | 561 | < 0.001 |
| **BCFA** | | | | | |
| **Isovalerian** | 8-10 | 9 | 11-12 | 11 | NS |
| **Isobutanoic** | 14-17 | 16 | 58-77 | 62 | < 0.001 |
| **Total** | 22-27 | 24 | 69-89 | 73 | < 0.001 |
| **Putrefactive SCFA** | 54-82 | 67 | 111-154 | 131 | < 0.001 |

*p* - analysis was based on U Mann-Whitney test. Statistical significance was established at $p < 0.05$.

current research confirmed one of the required guidelines for prebiotics, namely selectivity towards specific groups of bacteria [11, 14] and confirmed the possibility of using dextrin in a ready food product such as lactose-free milk. Also, the prebiotic index values for lactose-free milk enriched with dextrins indicates that *Lactobacillus* and *Bifidobacterium* were able to dominate the common environment of bacteria cultures in the tested milk. The prebiotic indexes after 24 and 48 hours of incubation for milk enriched with dextrin were higher than the values determined for this dextrin in cultures on microbiological substrates and significantly higher than prebiotic indexes determined by Olano-Martin et al. [21] for oligosaccharides (POS I, POS II). The reason for such good growth of lactic acid strains could be the availability of other sources of carbon, besides dextrin, that are naturally found in milk. The selective action of resistant dextrin and the rapid growth of *Lactobacillus* and *Bifidobacterium* strains resulted in lowering the pH of the product and this could further limit the growth of unfavorable strains, especially belonging to *Clostridium* genus.

Soluble fibers, which include a resistant dextrin from potato starch, are fermented by bacteria in the large intestine and are not digested and absorbed in the form of glucose into the bloodstream in the small intestine. Additionally, the soluble fibers produced by fermentation produce short-chain fatty acids such as butter, acetic and propionic acids. Recently, it has been revealed that short-chain fatty acids have a key effect on reducing the pH of intestine contents, stimulating the development of peripheral tissues (acetic acid), intestinal epithelium (butyric acid) and hepatocytes (propionic acid). The fermentation mechanism of compound formation reaching the final section of the digestive system depends on the strains that carry out the fermentation process, their enzymatic abilities and it also depends on the fermented substrate [8, 13, 20, 23]. The addition of dextrin to lactose-free milk did not modify the normal fermentation process, and the bacteria strains produced typical metabolites. In the presented studies, it was shown that the addition of dextrin to lactose-free milk increased the lactic acid concentration by 57% in relation to lactose-free milk without dextrin, which was also correlated with the higher number of lactic acid bacterial cells and a lower number of cells of bacteria strains preferring a higher pH such as *Clostridium* in the sample (MWL + D) compared to the MWL control sample. The total SCFA content was about 23% higher in lactose-free milk with the addition of potato starch dextrin than in the control without the addition of dextrin. Consumption of milk enriched with dextrin by people with lactose intolerance may contribute to increased absorption of calcium ions from the large intestine through increased production of short-chain fatty acids [8, 13, 20, 23].

As a positive fact in concentration of putrefactive SCFA and BCFA (sum of acids: valeric, isovaleric, isobutyric) is that it was significantly lower in MWL + D than in the MWL control group. This is a very important result because the reduced concentration of putrefactive SCFA indicates a reduction in the formation of unfavorable putrefactive processes in the intestine [2, 28].

## CONCLUSIONS

Resistant dextrin obtained from potato starch by simultaneous thermolysis and chemical modification with confirmed prebiotic properties added to lactose-free milk stimulates the growth of beneficial microorganisms (*Lactobacillus* and *Bifidobacterium*) and limits the growth of unfavorable strains especially from the *Clostridium* genus. Supplementation of lactose-free milk with resistant dextrin contributes to the increase of concentration of lactic acid, total concentration of short-chain fatty acids and in the reduction of putrefactive concentration. Intensive growth of *Lactobacillus* and *Bifidobacterium* strains, the production of acids especially lactic acid, and thus a decrease the pH in the tested milk contributed to obtaining the final product in the form of yogurt. Lactose-free milk supplemented with resistant dextrin from potato starch may favorably affect the intestinal microbiota system in people with lactose intolerance and it may reduce the digestive processes in the large intestine.

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