Appendix 3

Summary of Academic Accomplishments for the Purposes of Habilitation Procedure

Renata Barczyńska-Felusiak, PhD

1. Personal details

Full name: Renata Ewa Barczyńska-Felusiak, PhD
Place of work: Jan Dlugosz University in Czestochowa
Institute of Chemistry, Health and Food Sciences
Department of Dietetics and Food Research
ul: Armii Krajowej 13/15
42-200, Czestochowa, Poland
e-mail: r.barczynska-felusiak@ajd.czest.pl; r.barczynska@gmail.com

2. Diplomas and degrees held (provide a name, place and year of conferring and the title of PhD dissertation)

29.06.2005 Degree conferred: Bachelor of Biotechnology
Jan Dlugosz University in Czestochowa
Faculty of Matematics and Natural Sciences
Diploma paper title: "The use of new modified starches in food and nutrition"
Supervisor: Janusz Kapuśniak, PhD

29.06.2006 Degree conferred: Master of Environmental Protection
Jan Dlugosz University in Czestochowa
Faculty of Matematics and Natural Sciences
Diploma paper title: "The use of semicarbazide-modified starch and thiosemicarbazide for the trapping of heavy metal ions"
Supervisor: Janusz Kapuśniak, PhD

01.03.2011 Academic degree conferred: PhD in Technical Sciences in Biotechnology
Lodz University of Technology
Faculty of Biotechnology and Food Sciences
Title of the doctoral dissertation: "Resistant dextrins obtained from potato starch as substances with prebiotic properties"
Supervisor: professor Zdzisława Libudzisz
Janusz Kapuśniak, PhD, DSc, professor AJD

27.06.2016	Diploma of Postgraduate Education Dietary counseling - advances in human nutrition National Food and Nutrition Institute im. Prof. dra med. Aleksandra Szczygła in Warsaw

3. History of employment at research centers

01.10.2010 - 30.09.2012	Jan Dlugosz University in Czestochowa Institute of Chemistry, Environmental Protection and Biotechnology Department of Microbiology and Biotechnology Asistant
Since 01.04.2012	Jan Dlugosz University in Czestochowa Institute of Chemistry, Environmental Protection and Biotechnology since 01.10.2018r. Institute of Chemistry, Health and Food Sciences since 01.01.2016r. Department of Dietetics and Food Research Adjunct

4. Description of research accomplishment that is the basis of the habilitation application

The presented research accomplishment, as defined in Article 16(2) of the Act of March 14, 2003 on Academic Degrees and Academic Title and Degrees and Title in Arts (Journal of Laws 65, item 595 as amended), is a series of research papers.

a. Title of research accomplishment

Modification of the intestinal microbiota composition of children with overweight and obesity under the influence of dextrins from potato and corn starch.

b. Publications included in the achievement that is the basis for habilitation application.

H1 Barczynska R, Bandurska K., Slizewska K., Litwin M., Szalecki M., Libudzisz Z., Kapusniak J. (2015) Intestinal microbiota, obesity and prebiotics. Polish Journal of Microbiology 64(2), 93–100, **(IF_{2015}: 0,697*, MNiSW: 15pkt**)**.

H2 Barczynska R., Litwin M., Slizewska K., Szalecki M., Berdowska A., Bandurska K., Libudzisz Z., Kapusniak J. (2018) Bacterial microbiota and fatty acids in the feces of overweight and obese children. Polish Journal of Microbiology 67(3), 339–345, **(IF_{2017}: 0,784*, MNiSW: 15pkt**).**

H3 Barczynska R., Kapusniak J., Litwin M., Slizewska K., Szalecki M., Berdowska A., Bandurska K. (2014) Dextrins from potato starch as substances activating the growth of *Bacteroidetes* and *Actinobacteria* simultaneously inhibiting the growth of *Firmicutes*, responsible for the occurrence of obesity. Journal of Nutritional Ecology and Food Research 2(4), 340-347, **(IF_{2014}: 0*, MNiSW: 0pkt**).**

H4 Barczynska R., Kapusniak J., Litwin M., Slizewska K., Szalecki M. (2016) Dextrins from maize starch as substances activating the growth of *Bacteroidetes* and *Actinobacteria* simultaneously inhibiting the growth of *Firmicutes*, responsible for the occurrence of obesity. Plant Foods for Human Nutrition 71, 190-196, **(IF_{2016}: 2,368*, MNiSW: 35pkt**).**

H5 Barczynska R., Slizewska K., Litwin M., Szalecki M., Zarski A., Kapusniak J. (2015) The effect of dietary fiber preparations from potato starch on the growth and activity of bacterial strains belonging to the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*. Journal of Functional Foods 19, 661–668, **(IF_{2015}: 3,574*, MNiSW: 45pkt**).**

H6 Barczynska R., Slizewska K., Litwin M., Szalecki M., Kapusniak J. (2016) Efects of dietary fiber preparations made from maize starch on the growth and activity of selected bacteria from the *Firmicutes, Bacteroidetes,* and *Actinobacteria* phyla in fecal samples from obese children. Acta Biochimica Polonica 63, 1-6, **(IF_{2016}: 1,187*, MNiSW: 15pkt**).**

H7 Barczyńska R., Jurgoński A., Slizewska K., Juśkiewicz J., Kapuśniak J. (2017) Effects of potato dextrin on the composition and metabolism of the gut microbiota in rats fed standard and high-fat diets. Journal of Functional Foods 34, 398–407, **(IF_{2016}: 3,144*, MNiSW: 45pkt**).**

* impact factor (IF) is attributed according to year of publication, with 2017 IF used for papers published in 2018; data from Web of Science.
** MNiSW points are attributed in accordance with the list published by the Ministry of Science and Higher Education on January 26, 2017.

Co-authors' statements concerning their contributions to the publications comprising the monothematic series of publications are given in **Appendix 5**.

The research accomplishment that is the basis for habilitation application in the field of agricultural sciences has been presented in publications credited with **IF = 11.754 (170 MNiSW points).**

In addition, the applicant's other research has been presented in publications with **IF = 16.26 (276 MNiSW points)**

Based on the Journal Citation Reports (JCR) index, the overall impact factor according to year of publication is **IF = 28.014, and the overall sum of MNiSW points is 446.**

The number of citations according to the Web of Science (WoS) database is **94 (without self-citations 74)**

Hirsch index according to the Web of Science (WoS) database: **6**

c. Discussion or the research objectives of the aforementioned publications and their results, including potential applications.

The gut microbiota is crucial to the normal functioning of the human body, as it affects, amongst others, metabolic processes as well as immunological and physiological functions (Neish, 2002; Stewart et al., 2004; Nicholson et al., 2005; Walker and Lawley 2013; Milani et al., 2016; Zhernakova et al., 2016). In recent years, researchers have focused on the role of the gut microbiota in the development of chronic diseases, such as obesity, type 2 diabetes mellitus, inflammatory bowel disease, and colorectal cancer (Tamboli et al., 2004; Backhed et al., 2004; Backhed et al., 2007; Tannock, 2008; Feng et al., 2010; De Filippo et al., 2010; DuPont i DuPont, 2011; Milani et al., 2016; Lim et al., 2017; Vandeputte et al., 2017a).

Obesity, which has been termed an epidemic of the 21st century, is associated with both environmental and genetic factors. According to reports of the Polish National Food and Nutrition Institute, the proportion of overweight and obese children and adolescents in Poland increased from 9% to over 11% from 1995 to 2000. Currently, almost 16% of children and adolescents in Poland are either overweight or obese. These data are corroborated by the World Health Organization (WHO), according to which the number of overweight children in Poland has increased threefold. In Europe, every fourth child is obese. In children and adolescents, obesity is a risk factor for serious diseases, including diabetes mellitus as well as cardiovascular, skeletal, and metabolic disorders.

Numerous studies have shown that obesity is largely associated with, amongst others, changes in the composition and metabolic function of the gut microbiota. It is thought that of particular importance is maintaining the right proportion between strains belonging to the phyla *Bacteroidetes* and *Firmicutes* (Ley et al., 2006; Sanz and Santacruz, 2008). Studies by Backhed et al., Gordon et al., and De Filippo et al. have revealed that the most abundant strains in obese individuals are *Firmicutes* bacteria (approx. 60–80%), with a much smaller proportion of *Bacteroidetes,* as compared to lean individuals (Backhed et al., 2004; Ley et al., 2006; Backhed et al., 2007; De Filippo et al., 2010; Canfora et al., 2015; Lu et al., 2016; Raza et al., 2017).

Diet directly affects the composition and activity of the gut microbiota, which in turn influences the development of overweight and obesity. Therefore, in my research I investigated the application of preparations (substances, compounds) that could be used as supplements to stimulate the growth of beneficial microorganisms, while being well-tolerated by humans. In

particular I focused on starch-based products, such as dietary fiber preparations form potato and corn starch, proving their prebiotic properties both *in vitro* and *in vivo*.

According to Roberfroid et al. (2010), prebiotics are defined as substances promoting "the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host". Recently, Gibson et al. (2017) have updated that definition to "a substrate that is selectively utilized by host microorganisms conferring a health benefit." The effectiveness of prebiotics is attributable to the fact that they are not hydrolyzable or absorbed in the upper part of the gastrointestinal tract, and so they pass to the colon in an unaltered form, where they provide nutrients to beneficial bacteria (Roberfroid, 2002). The health benefits conferred by prebiotics derive from their effects, such as: inhibition of the development of pathogens due to the stimulation of fermenting microorganisms, decreased pH, shorter intestinal transit time, accelerated metabolism, lower cholesterol levels and glycemic response, reduced demand for energy, increased bioavailability of mineral components, prevention of so-called traveler's diarrheas, and reduced risk of gastrointestinal diseases, including tumors (FAO; Vernazza et al., 2006; Roberfroid, 2007; Slavin, 2013; Beserra et al., 2015; Rastall & Gibson, 2015; Christodoulides et al., 2016; Fernandes et al., 2017; Hume et al., 2017; Nicolucci & Reimer, 2017; Vandeputte et al., 2017b). The mechanism of action of prebiotics is complex as they influence the biochemical processes occurring in the intestines, increase the number of beneficial bacteria (mostly saccharolytic) competing with pathogens, block receptors in the intestinal mucosa preventing adhesion by pathogenic bacteria, and supply the colonocytes with nutrients in the form of SCFA resulting from prebiotic fermentation (Rastall et al., 2005; Ouwehand et al., 2005; Hutkins et al., 2016; Koh et al., 2016). I have described the properties of prebiotics and their mechanism of action in the following publications: Śliżewska et al., 2013; Barczynska, 2014; Kapusniak et al., 2014; Barczynska et al., 2015.

The objectives of the studies comprising the research accomplishment were to establish the composition and proportions of microorganisms in feces from overweight and obese Polish children, to check whether these parameters are different from those identified in normal-weight Polish children, and to determine whether dextrins from potato or corn starch may stimulate the growth of the prevalent strains of enteric bacteria: *Bacteroides*, *Prevotella* (*Bacteroidetes*) and *Bifidobacterium* (*Actinobacteria*), and inhibit *Clostridium* and *Lactobacillus* (*Firmicutes*), thus modifying the proportions of the bacteria considered crucial to preventing and managing overweight and obesity in children. The adopted objectives were attained through laboratory studies involving *in vitro* methods and *in vivo* experiments on rats.

The objectives of my research included:

1. To delineate the current state of knowledge about the role of gut microbiota in maintaining normal body weight and in obesity prevention and treatment.
2. To determine the quantity and proportions of the bacterial genera prevailing in the human intestines, that is: *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Clostridium*, *Lactobacillus* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*) in feces from 10 overweight (BMI 25.68–29.48) and 10 obese (BMI 31.71–41.18) Polish children aged 5 to 15 years. The control group consisted of 20 normal-weight children (BMI 18.5–22.38).
3. To establish whether the main genera of enteric bacteria, that is, *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Clostridium*, *Lactobacillus* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*), isolated from the feces of overweight and obese children, can utilize as the only source of carbon potato and corn dextrins, and to determine the effects of those dextrins on the quantity and proportions of those strains in co-cultures.
4. To determine the types and concentrations of fermentation products from bacteria isolated from the feces of overweight, obese, and normal-weight children, in the media containing potato or corn dextrins.
5. To verify whether potato and corn dextrins may stimulate the growth of the bacterial genera prevailing in the human intestines, that is *Bacteroides*, *Prevotella* (*Bacteroidetes*) and *Bifidobacterium* (*Actinobacteria*), while reducing the growth of *Clostridium* and *Lactobacillus* strains (*Firmicutes*) (*in vitro* studies were conducted directly in feces from children).
6. To elucidate the effects of potato dextrins fed to rats in low- and high-fat diets on the quantity and proportions of the main enteric bacterial genera: *Bacteroides*, *Prevotella*, *Clostridium*, *Lactobacillus*, and *Bifidobacterium* (*in vivo* studies on rats).
7. To analyze the basic functional parameters of the rat gastrointestinal system: lactic acid production, the concentration and types of short-chain fatty acids, and glycolytic activity in the cecal digesta of rats fed low- and high-fat diets supplemented with potation dextrin (*in vivo* studies on rats).

In my studies, I used two resistant dextrins from potato starch and two from corn starch, obtained by simultaneous thermolysis and chemical modification in the presence of an inorganic acid (hydrochloric acid) as a dextrinization catalyst and an organic acid (citric or tartaric acid) as a modifier. The process of producing resistant dextrins from potato starch was patented (PL.220965 Jochym et al., 2015 and PL.221497 Kapuśniak et al., 2015). The manner of producing corn dextrins was analogous, and differed only in heating time (Jochym et al.,

2012). These resistant dextrins are thus recently developed and patented substances, produced at the Department of Dietetics and Food Research, Jan Dlugosz University in Czestochowa.

The applied dextrins were studied previously (Barczyńska, 2010, Barczynska et al., 2012, 2014, 2015) and it was found that the solubility of dextrins from potato starch was 63–68%, while that of dextrins from corn starch was 95%. The weight-average molecular mass (M_w) of potato dextrins was 1828 g/mol (mean DP = 11 for dextrins modified with tartaric acid) and 4.8 $*10^3$ g/mol (mean DP = 25–30 for dextrins modified with citric acid). The main fraction of corn dextrins modified with citric acid had a mean DP = 20, accounting for 92% of the total, while the main fraction of dextrins modified with tartaric acid had a mean DP = 18, accounting for 85% of the total. The modification of potato and corn starch with citric or tartaric acid led to an increased content of the indigestible fraction up to 65–70% (Barczyńska, 2010, Jochym et al., 2012). *In vitro* studies of the effects of these dextrins on strains from culture collections showed their prebiotic properties due to the stimulation of beneficial bacteria (*Lactobacillus* and *Bifidobacterium*) and inhibition of the proliferation of pathogens (*Clostridium*). As a result of the fermentation of these dextrins, the beneficial gut bacteria produced *in vitro* higher amounts of lactic acid and SCFA (Barczyńska, 2010; Barczynska et al., 2012, 2014, 2015).

1. The role of the gut microbiota in maintaining normal weight and its effects on the prevention and treatment of obesity

A literature review enabled me to identify the latest developments concerning the effects of the gut microbiota on maintaining normal weight and its potential in terms of obesity prevention and treatment. These data were presented in publication **H1: Barczynska R, Bandurska K., Slizewska K., Litwin M., Szalecki M., Libudzisz Z., Kapusniak J. (2015) Intestinal microbiota, obesity and prebiotics. Polish Journal of Microbiology 64(2), 93–100, (IF_{2015}: 0,697, MNiSW: 15pkt).**

The literature data indicate that the development of overweight and obesity is closely correlated with changes in the composition of the gut microbiota. Overweight and obese individuals exhibit a preponderance of bacterial strains belonging to the phylum *Firmicutes*, with a much smaller presence of *Bacteroidetes*, while in normal-weight individuals these proportions are reversed. However, the mechanisms in which the gut microbiota may contribute to or prevent obesity have not been fully elucidated. It has been found that obesity is associated with high plasma levels of lipopolysaccharide (LPS), which are components of the cell walls of Gram-negative bacteria. Another potential major contributor to obesity is alkaline phosphatase (IAP), which helps to degrade lipids derived from food and plays a

significant role in LPS detoxification. It has been shown that IAP expression may be controlled by the gut microbiota. Yet another factor linking the gut microbiota to obesity is the fact that the former blocks of the expression of the fasting-induced adipose factor (FIAF). FIAF inhibits the activity of lipoprotein lipase (LPL), an enzyme responsible for the storage of energy in the form of fat. Decreased FIAF expression leads to increased LPL activity and greater fat production. Evidence shows that the gut microbiota modulates the activity of the endocannabinoid system, thus affecting the intestinal barrier function. Due to the fact that **to date the role of the gut microbiota in maintaining normal-weight has not been fully elucidated, with many inconclusive or conflicting research results, and given the absence of literature reports on the gut microbiota in overweight and obese Polish children, I decided to address these issues and determine the proportions of the most abundant enteric bacteria, that is, *Bacteroides* and *Prevotella* belonging to the phylum *Bacteroidetes* as well as *Clostridium* and *Lactobacillus* belonging to the phylum *Firmicutes* in feces from overweight children (BMI 25.68–29.48), obese children (BMI 31.71–41.18), and normal-weight children (BMI 18.5–22.38). Moreover, I monitored the quantity of bifidobacteria (*Actinobacteria*), which are extremely beneficial to children's health.**

2. **The quantity and proportions of the most abundant enteric bacteria of the genera *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Clostridium*, *Lactobacillus* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*) in feces from 10 overweight (BMI 25.68–29.48) and 10 obese (BMI 31.71–41.18) Polish children aged 5–15 years. The control group consisted of 20 normal-weight (BMI 18.5–22.38) children.**

My studies on the proportions of *Firmicutes, Bacteroidetes,* and *Actinobacteria* strains in feces from overweight (BMI 25.68–29.48), obese (BMI 31.71–41.18), and normal-weight (BMI 18.5–22.38) children are presented in publication **H2**: **Barczynska R, Litwin M., Slizewska K., Szalecki M., Berdowska A., Bandurska K., Libudzisz Z., Kapusniak J. (2018) Bacterial microbiota and fatty acids in the feces of overweight and obese children. Polish Journal of Microbiology 67(3), 339–345, (IF_{2017}: 0,784, MNiSW: 15pkt).**

I analyzed feces from a representative group of overweight, obese, and normal-weight children aged 5–15 years selected on the basis of a questionnaire administered to the patients of the Children's Memorial Health Institute in Warsaw. The selection of that age group was

dictated by the recent increase in obesity in children. The study material was feces, as it best reflects the *in vivo* conditions of the colon, it is easily accessible, and enables many original results. I determined the quantity and proportions of bacterial strains of the genera *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Lactobacillus*, *Clostridium* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*) in the feces of overweight, obese, and normal-weight children. The bacteria were identified by fluorescence *in situ* hybridization (FISH). The classification of the identified strains to the studied genera and phyla was confirmed by 16S rRNA reactions. In addition, I checked for any relationships between the presence of the various bacterial strains in the feces of overweight/obese children and their diet and environmental factors. I also determined lactic acid content as well as the concentration and types of SCFA and BCFA in the feces.

I found that among the five studied bacterial genera, the most abundant strains in feces from children with overweight and obesity belonged to the genus *Clostridium*, with a mean cell count of 8.03 $\log_{10}$ CFU/g (in feces from normal-weight children, the count of these bacteria was lower by approx. 14%). In turn, the most abundant bacterial genera in feces from normal-weight children were *Bacteroides* (8.57 $\log_{10}$ CFU/g) and *Bifidobacterium* (9.07 $\log_{10}$ CFU/g). The counts of those bacteria were lower in feces from overweight and obese children by approx. 20% and 18%, respectively. Furthermore, *Prevotella* counts were by 30% lower in overweight and obese children as compared to normal-weight children. The cell counts of *Lactobacillus* strains in feces from overweight/obese children and from those with normal weight were similar: 7.77 $\log_{10}$ CFU/g and 7.81 $\log_{10}$ CFU/g, respectively. It was found that in feces from overweight and obese children, *Clostridium* and *Lactobacillus* strains (*Firmicutes*) constituted the majority of the bacterial population (with a mean of 45.9%), with *Clostridium* being the most abundant genus, while *Bacteroides* and *Prevotella* (*Bacteroidetes*) accounted for 32.4%, and *Bifidobacterium* (*Actinobacteria*) for 21.7% of the total. In feces from overweight and obese children, the proportions of the main bacterial strains differed from those in normal-weight children. In feces from normal-weight children, the proportions of *Bacteroides* and *Prevotella* strains (*Bacteroidetes*) and *Clostridium* and *Lactobacillus* (*Firmicutes*) were similar at approx. 39% of the total bacterial populations, with *Actinobacteria* accounting for the remaining 22%. A particularly significant increase in *Firmicutes* (mostly *Clostridium*) with a concomitant decrease in *Bacteroidetes* was observed in feces from extremely obese children (obesity III^0, with BMI of 40.1 and mean BMI-SDS of 2.4), in whom *Clostridium* and *Lactobacillus* accounted for 54%, *Bacteroides* and *Prevotella* for 25%, and *Bifidobacterium* for 21% of the total. This is an important finding as it **supports the hypothesis that the gut microbiota, also of Polish children, can serve as a marker of obesity**. My research on the dietary habits and lifestyles of overweight and obese children showed that they often

consumed large amounts of red meat and exhibited low physical activity with passive behavior during leisure time. I also found that 95% of overweight and obese children had obese parents. The concentrations of SCFA, BCFA, and lactic acid were lower by 34%, 18%, and 40%, respectively, in feces from overweight/obese children as compared to normal-weight children. **These were the first studies on the composition and proportions of the main enteric bacterial strains in feces from overweight, obese, and normal-weight Polish children.**

It was found that **the most abundant bacteria in feces from overweight and obese children are *Clostridium* (*Firmicutes*), with smaller populations of *Bacteroides* and *Prevotella* strains (*Bacteroidetes*), while in feces from normal-weight children these proportions are reversed. Thus, the gut microbiota may serve as a marker for overweight and obesity. In addition, since feces from obese children contained fewer bifidobacteria (*Actinobacteria*), they might also be considered a marker for obesity.**

As many as 100 strains were isolated from the feces of 10 overweight and 10 obese children; those strains were then classified into genera and phyla by means of genetic methods. The genera *Lactobacillus*, *Bifidobacterium*, *Prevotella*, *Bacteroides*, and *Clostridium* were represented by 20 strains each. I also isolated 100 *Lactobacillus*, *Bifidobacterium*, *Prevotella*, *Bacteroides*, and *Clostridium* strains from the feces of 20 normal-weight children. Using the large collection of strains isolated from the feces of overweight/obese and normal-weight children, in the next stage of research I investigated the ability of these bacteria to utilize the studied dextrins as a sole source of carbon.

3. **The ability of *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Clostridium*, *Lactobacillus* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*) isolated from the feces of overweight/obese and normal-weight children to utilize potato and corn dextrins as the sole source of carbon, and the effect of those dextrins on the quantity and proportions of the most abundant strains of the isolated gut bacteria in co-cultures.**

Findings from these studies were presented in two papers: **H3** and **H4:**
H3: Barczynska R., Kapusniak J., Litwin M., Slizewska K., Szalecki M., Berdowska A., Bandurska K. (2014) Dextrins from potato starch as substances activating the growth of *Bacteroidetes* and *Actinobacteria* simultaneously inhibiting the growth of *Firmicutes*, responsible for the occurrence of obesity. Journal of Nutritional Ecology and Food Research 2(4), 340-347, (IF_{2014}: 0, MNiSW: 0pkt.).

H4: Barczynska R., Kapusniak J., Litwin M., Slizewska K., Szalecki M. (2016) Dextrins from maize starch as substances activating the growth of *Bacteroidetes* and *Actinobacteria* simultaneously inhibiting the growth of *Firmicutes*, responsible for the occurrence of obesity. Plant Foods for Human Nutrition 2016, 71, 190-196, (IF_{2016}: 2,368, MNiSW: 35pkt).

It was found that irrespective of the type of dextrins and methods of their production, the isolated strains used them as a sole source of carbon to a similar extent.

In co-cultures of the isolated bacterial strains in media containing potato dextrins as the only source of carbon, dextrins were best utilized by *Lactobacillus* and *Bifidobacterium* strains (8.28 and 8.6 $\log_{10}$ CFU/mL) irrespective of whether they were isolated from the feces of overweight/obese or normal-weight children. Potato dextrins were utilized less efficiently by *Bacteroides*, *Clostridium*, and *Prevotella* as their counts ranged from 7.59 to 7.94 $\log_{10}$ CFU/mL. Only in media containing potato dextrin modified with citric acid, co-cultured *Bacteroides* strains isolated from the feces of overweight and obese children were found to grow slightly slower (by 6%) than those isolated from the feces of normal-weight children.

Corn dextrin was utilized most efficiently by *Lactobacillus* and *Bifidobacterium* strains (8.28–8.54 $\log_{10}$ CFU/mL) and least efficiently by *Bacteroides* isolated from the feces of both overweight/obese and normal-weight children (7.58–7.88 $\log_{10}$ CFU/mL). *Bifidobacterium* strains isolated from the feces of overweight and obese children grew more actively in the media containing dextrin modified with tartaric acid as compared to *Lactobacillus* and *Bifidobacterium* strains isolated from the feces of normal-weight individuals. The counts of *Prevotella* and *Clostridium* strains isolated from the feces of overweight and obese children were similar, ranging from 7.83 to 8.13 $\log_{10}$ CFU/mL, while those from normal-weight children ranged from 7.90 to 8.20 $\log_{10}$ CFU/mL.

I found very similar proportions of the studied *Bacteroidetes* (*Bacteroides*, *Prevotella*), *Firmicutes* (*Lactobacillus*, *Clostridium*) and *Actinobacteria* (*Bifidobacterium*) strains cultured in media containing potato and corn dextrins, irrespective of the type of dextrin applied and of the origin of the strains (feces of overweight/obese or normal-weight children). In cultures with both potato and corn dextrins *Firmicutes* strains accounted for 40–41%, *Bacteroidetes* for 38–39%, and *Actinobacteria* for 21–22% of the entire bacterial population.

It was found that **the method of producing potato and corn dextrins, including the organic acids used (citric, tartaric), did not lead to significant differences in the growth and proportions of the studied *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Lactobacillus*,**

***Clostridium* (*Firmicutes*) and *Bifidobacterium* (*Actinobacteria*) strains, which indicates that the dextrins were utilized as a carbon source by the strains to a similar degree.**

4. Fermentation products of bacteria isolated from the feces of overweight, obese, and normal-weight children and cultured in media with potato or corn dextrins

Since I did not find any significant differences in the utilization of corn and potato dextrins as a sole source of carbon by *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Lactobacillus*, *Clostridium* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*) strains, I proceeded to determine whether the isolated strains fermented the studied dextrins in the same way. To that end, lactic acid content as well as the concentrations and types of SCFA and BCFA were determined in co-cultures of the strains isolated from overweight/obese and normal-weight children, in media containing potato or corn dextrins.

It was found that the mean lactic acid content in co-cultures of bacteria isolated from the feces of overweight and obese children was by 50% and 41% lower (in media with potato and corn dextrins modified with citric acid, respectively) by 49% and 34% lower (in media with potato and corn dextrins modified with tartaric acid, respectively) than that in co-cultures of isolates from normal-weight children. Similarly, the concentration of SCFA was lower by 45% and 43% (in media with potato and corn dextrins modified with citric acid, respectively) and by 48% and 39% (in media with potato and corn dextrins modified with tartaric acid, respectively) in cultures of strains isolated from the feces of overweight and obese children as compared to normal-weight individuals. Furthermore, when the growth media contained dextrins, the concentration of acetic, butyric, and valeric acids in cultures of bacteria isolated from overweight and obese children was half of that found for bacteria isolated from the feces of normal-weight children. Finally, the concentration of BCFA in cultures of bacteria isolated from the feces of overweight/obese children was lower by 64% and 74% (in media with potato and corn dextrins modified with citric acid, respectively) and by 73% and 54% (in media with potato and corn dextrins modified with tartaric acid, respectively) as compared to bacteria isolated from normal-weight children.

Bacteroides, *Prevotella* (*Bacteroidetes*), *Lactobacillus*, *Clostridium* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*) isolated from the feces of overweight and obese children metabolized potato and corn dextrins producing metabolites typical of the various strains. Despite the fact that bacteria isolated from the feces of both overweight/obese and normal-

weight children utilized dextrins as a source of carbon to the same extent, they did so with a varying degree of intensity. SCFA and BCFA concentrations were higher in media containing potato dextrin vs. corn dextrin; higher in media containing dextrins modified with citric acid vs. tartaric acid; and also higher in cultures of strains isolated from the feces of normal-weight vs. overweight/obese children. It should be noted that **this was the first report about the ability of *Bacteroidetes, Firmicutes*, and *Actinobacteria* enteric strains to utilize potato or corn dextrins as a source of carbon.**

5. Growth stimulation of the main enteric bacterial strains: *Bacteroides*, *Prevotella* (*Bacteroidetes*) and *Bifidobacterium* (*Actinobacteria*), and inhibition of *Clostridium* and *Lactobacillus* (*Firmicutes*) by potato and corn dextrins (*in vitro* studies conducted directly in feces from children).

Since it was found that *Bacteroides*, *Prevotella*, *Lactobacillus*, *Clostridium*, and *Bifidobacterium* strains, irrespective of who they were isolated from, could utilize potato and corn dextrins as a source of carbon, the logical next step was to investigate whether the dextrins could stimulate the growth of *Bacteroidetes* and *Actinobacteria* strains while inhibiting *Firmicutes* strains. Cultures were conducted directly in fecal samples from overweight and obese children with the addition of dextrins (the control samples did not contain dextrins). The studied bacteria were enumerated using the plate count method and FISH; also lactic acid content and the concentrations of SCFA and BCFA were determined.

The findings were presented in paper **H5: Barczynska R., Slizewska K., Litwin M., Szalecki M., Zarski A., Kapusniak J. (2015) The effect of dietary fiber preparations from potato starch on the growth and activity of bacterial strains belonging to the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*. Journal of Functional Foods 19, 661–668, (IF_{2015}: 3,574, MNiSW: 45pkt).**

It was found that potato dextrins stimulated the growth of *Bacteroides*, *Prevotella*, and *Bifidobacterium* strains (belonging to the phyla *Bacteroides* and *Actinobacteria*, respectively), while inhibiting the growth of *Clostridium* strains (*Firmicutes*).

The addition of dextrins to fecal samples from overweight and obese children led to an increase in *Bacteroides* (*Bacteroidetes*) by 17% and *Bifidobacterium* (*Actinobacteria*) by 15%, and a slight decline in *Prevotella* (*Bacteroidetes*) by 3%, both in cultures containing dextrins modified with citric and tartaric acid as compared to feces incubated without dextrins. The presence of dextrins resulted in a decrease in the counts of *Clostridium* (*Firmicutes*) on

average by 36% (for fecal samples incubated with dextrins modified with citric acid) and by 55% (for tartaric acid modification) as compared to fecal samples incubated without dextrins. The counts of lactobacilli (*Firmicutes*) rose on average by 21% after feces incubation with dextrins modified with citric acid and by 10% after incubation with dextrins modified with tartaric acid as compared to fecal samples incubated without dextrins. In summary, following the incubation of fecal samples from overweight and obese children in media containing dextrins modified with citric acid, *Bacteroidetes* strains accounted for 35%, *Actinobacteria* for 25%, and *Firmicutes* for 35% of the total bacterial population. In turn, after the incubation of fecal samples from overweight and obese children in media containing dextrins modified with tartaric acid, *Bacteroidetes* strains accounted for 38%, *Actinobacteria* for 26%, and *Firmicutes* for 36% of the total bacterial population.

The incubation of feces from overweight and obese children in media containing potato dextrins led to an increased production of lactic acid, SCFA, and BCFA. Following the incubation of fecal samples from overweight and obese children in media containing dextrin modified with citric acid, lactic acid content was higher by 40% as compared to incubation with dextrin modified with tartaric acid. In turn, the total SCFA concentration was higher by 10% after the incubation of feces from overweight and obese children in media containing dextrins modified with tartaric acid vs. citric acid.

Dextrins modified with tartaric acid clearly stimulated the growth of *Bacteroides* and *Bifidobacterium* strains while inhibiting *Clostridium*; in addition feces incubated in media containing these dextrins revealed more SCFA as compared to those modified with citric acid.

This is an important finding as it is the first report indicating the ability of dextrins obtained from potato starch to selectively stimulate the growth of *Bacteroides* and *Bifidobacterium* strains, which are more abundant in feces from normal-weight children.

Similar studies were performed for corn dextrins, with the results presented in paper **H6: Barczynska R., Slizewska K., Litwin M., Szalecki M., Kapusniak J. (2016) Efects of dietary fiber preparations made from maize starch on the growth and activity of selected bacteria from the *Firmicutes, Bacteroidetes,* and *Actinobacteria* phyla in fecal samples from obese children. Acta Biochimica Polonica 63, 1-6, (IF_{2016}: 1,187, MNiSW: 15pkt).**

The proportions of *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Clostridium*, *Lactobacillus* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*) strains after the incubation of fecal samples from overweight and obese children in media containing corn dextrins were slightly modified as compared to feces incubated without dextrins. Following the incubation of feces from overweight and obese children in media containing dextrins modified with either citric or tartaric acid, *Lactobacillus* and *Clostridium* strains taken together accounted for 46% and 44% of the

entire bacterial population, respectively. The proportion of *Prevotella* and *Bacteroides* taken together accounted for only 33% and 35% of the gut microbiota after the incubation of feces from overweight and obese children in media containing dextrins modified with citric and tartaric acid, respectively. The proportions of *Bifidobacterium* remained similar at 21%. The addition of corn dextrins (irrespective of how they were produced) to fecal samples obtained from overweight and obese children did not significantly affect the counts of the analyzed *Firmicutes, Bacteroidetes*, and *Actinobacteria* strains.

The concentration of lactic acid, SCFA, and BCFA were much higher after the incubation of fecal samples from overweight and obese children in media containing corn dextrins as compared to the control media. It was found that after the incubation of feces from overweight and obese children in media containing dextrins modified with tartaric acid, SCFA content was twice as high as in media containing dextrins modified with citric acid, while the concentrations of lactic acid and BCFA remained similar.

The findings presented in publications H2–H6 showed that potato and corn dextrins provided a source of carbon for Bacteroidetes (*Bacteroides*, *Prevotella*), *Actinobacteria* (*Bifidobacterium*), and *Firmicutes* (*Lactobacillus*, *Clostridium*) strains isolated from the feces of both overweight/obese and normal-weight children. Potato dextrins acted selectively, stimulating the growth of *Bacteroidetes* and *Actinobacteria* strains, while inhibiting *Firmicutes*, and in particular *Clostridium*, strains. This selective activity was not found for corn dextrins. As both potato and corn dextrins stimulated the growth of saccharolytic strains, they led to higher SCFA and lactic acid concentrations.

6. The effects of potato dextrins fed to rats in low- and high-fat diets on the abundance and relative proportions of the predominant enteric bacterial strains belonging to the genera *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Clostridium*, *Lactobacillus* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*). *In vivo* studies on rats.

According to my research design, dextrins which constitute a source of carbon for the bacteria isolated from the feces of overweight/obese and normal-weight children, which stimulate the growth of *Bacteroides* and *Prevotella (Bacteroidetes*) and *Bifidobacterium* (*Actinobacteria*) strains while inhibiting *Clostridium* (*Firmicutes*), should be subjected to *in vivo* studies. For the next stage of my research, I selected dextrins obtained from potato starch. Given that in the future dextrins may be used as a dietary component, I decided to study dextrins modified with citric acid, which is generally considered safe and is widely used as an acidity regulator and preservative. *In vivo* studies, fundamental for predicting the potential

usefulness of potato dextrins in humans, were conducted on 32 Wistar rats, which are typically used in nutritional research.

For 12 weeks the rats were given either low- or high-fat diets supplemented with potato dextrin (5% of the total dietary components); the controls were low- or high-fat diets without dextrin. The diets were prepared in such a way as to establish the effects of dextrins on growth stimulation of selected *Bacteroides*, *Prevotella*, *Clostridium*, *Lactobacillus*, and *Bifidobacterium* strains in feces from rats consuming low- or high-fat diets. The microbiota composition of rat feces was studied using the plate count method and FISH.

The findings were presented in paper **H7: Barczyńska R., Jurgoński A., Slizewska K., Juśkiewicz J., Kapuśniak J. (2017) Effects of potato dextrin on the composition and metabolism of the gut microbiota in rats fed standard and high-fat diets. Journal of Functional Foods 34, 398–407, (IF_{2016}: 3,144, MNiSW: 45pkt).**

Feces from rats fed both low- and high-fat diets with potato dextrin revealed beneficial effects in the form of increased *Bacteroides*, *Prevotella*, and *Bifidobacterium* counts combined with a slight decrease in *Lactobacillus* and a significant decline in *Clostridium* after 9 weeks of experiment. These proportions became even more favorable after 12 weeks of feeding, with a marked preponderance of *Bacteroides* and *Prevotella* (*Bacteroidetes*) strains over *Clostridium* (*Firmicutes*) strains; there was also an increase in the proportion of *Bifidobacterium* (*Actinobacteria*). In feces from rats fed a low-fat diet with potato dextrin, *Bacteroidetes* strains accounted for 48.44% of the total bacterial population, *Firmicutes* accounted for 30.13% (with *Lactobacillus* comprising 53% of *Firmicutes*), and *Actinobacteria* accounted for 21.43%; in the case of rats consuming a high-fat diet with dextrin, *Bacteroidetes* accounted for 42.53%, *Firmicutes* for 35.24% (with *Lactobacillus* comprising 56% of *Firmicutes*), and *Actinobacteria* for 22.23% of the total. In the control groups of rats fed low- and high-fat diets without potato dextrin, the proportions of the various bacterial phyla in weeks 9 and 12 were similar. *Bacteroides* and *Prevotella* strains taken together accounted for approx. 38–39% of the total bacterial population, *Clostridium* and *Lactobacillus* strains accounted for approx. 39–40%, while *Bifidobacterium* for approx. 20–21%.

The *in vivo* results indicate that **potato dextrin modified with citric acid, as a component of both low- and high-fat diets, stimulated the growth of *Bacteroides*, *Prevotella* (*Bacteroidetes*) and *Bifidobacterium* (*Actinobacteria*) strains, while inhibiting *Firmicutes*, and especially *Clostridium*, strains.**

It was found that after 12 weeks of experiment, dietary intake decreased in the groups of rats fed low- and high-fat diets with dextrin as compared to the controls; the lowest intake

was recorded for the group fed a high-fat diet with dextrin. The highest body weight gain was observed for rats consuming the high-fat control diet; the addition of potato dextrin to that diet led to a 5% decrease in body weight and a 12% decrease in adipose tissue as compared to rats fed diets without dextrin. The lowest body weight gain was seen in the group of rats consuming the low-fat diet. The addition of dextrin to that diet resulted in an 8% increase in body weight as compared to rats fed the low-fat diet without dextrin.

The critical findings were that the supplementation of low- and high-fat diets with potato dextrin modified with citric acid led to a marked increase in, and indeed a clear predominance of *Bacteroidetes* (*Bacteroides*, *Prevotella*) and *Actinobacteria* (*Bifidobacterium*) strains over *Firmicutes* (*Clostridium*) strains and that that resulted in a lower dietary intake by rats fed both low- and high-fat diets containing dextrin and in decreased body weight and adipose tissue content in rats consuming the high-fat diet with dextrin. My studies within this scope are fully original.

7. The basic gastrointestinal function indicators in rats, the production of lactic acid, the concentration and types of SCFA, and glycolytic activity in the cecal digesta of rats fed low- and high-fat diets supplemented with potato dextrin. *In vivo* studies on rats.

The findings were presented in paper **H7: Barczyńska R., Jurgoński A., Slizewska K., Juśkiewicz J., Kapuśniak J. (2017) Effects of potato dextrin on the composition and metabolism of the gut microbiota in rats fed standard and high-fat diets. Journal of Functional Foods 34, 398–407, (IF_{2016}: 3,144, MNiSW: 45pkt).**

The supplementation of low- and high-fat diets with potato dextrin had a substantial influence on selected parameters of the small intestine and cecum. The supplementation of the low-fat diet significantly increased the weight of the small intestine and viscosity of its digesta. The relative weight of the cecum and its digesta also increased in the presence of dextrin. In the case of the colon, the weight of its digesta decreased in the presence of potato dextrin both in low- and high-fat diets. Furthermore, both low- and high-fat diets with dextrin lowered the pH of small intestine digesta by 0.5–1 units and that of the cecum and colon by 0.1–0.4 units as compared to the controls.

In my research, I found that the gut microbiota of rats metabolized resistant dextrin obtained from potato starch, as indicated by increased glycolytic activity, and especially α- and β-glucosidase and α- and β-galactosidase activity, in cecal digesta. The supplementation of diets with potato dextrin also increased the production of SCFA in the ileum (more so in the

case of the low-fat diet), which was correlated with a greater presence of glycolytic bacteria. Another beneficial result was the fact that the concentration of BCFA (sum of valeric, isovaleric, and isobutyric acids resulting from protein decomposition) was much lower in the group fed the high-fat diet supplemented with potato dextrin than in the other groups (half of what was found in rats consuming the low-fat diet with dextrin). This is a significant finding in that it indicates a reduction in unfavorable putrefactive processes in the intestines.

I was the first to show that potato dextrin reduces dietary intake and leads to changes in the ileum that may mitigate overweight and obesity. At a time of a raging epidemic of overweight and obesity both in children and adults and the spread of bad dietary habits, I believe it is a major accomplishment indicating that the studied dextrin may be successfully used in the prevention of overweight and obesity in humans. My studies within this scope are fully original.

The most important accomplishments from the research described in the presented series of publications (H1–H7) are the following findings:

1. The profiles of the gut microbiota isolated from the feces of overweight (BMI 25.68–29.48), obese (BMI 31.71–41.18), and normal-weight Polish children (BMI 18.5–22.38) differ markedly. The predominant enteric bacteria in overweight and obese children are *Clostridium* strains belonging to the phylum *Firmicutes*, with a smaller proportion of *Bacteroides* and *Prevotella* (*Bacteroidetes*) strains and *Bifidobacterium* strains (*Actinobacteria*). On the other hand, the main bacteria in feces from normal-weight children are *Bacteroides*, *Prevotella*, and *Bifidobacterium* strains, with a minor proportion of *Clostridium* strains. The abundance of *Lactobacillus* strains is similar in overweight/obese and normal-weight children.
2. In feces from overweight and obese children, the concentrations of short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), and lactic acid are lower by 34%, 18%, and 40% as compared to feces from normal-weight children.
3. *Bacteroides* and *Prevotella* (*Bacteroidetes*), *Lactobacillus* and *Clostridium* (*Firmicutes*), as well as *Bifidobacterium* (*Actinobacteria*) isolated from the feces of overweight, obese, and normal-weight children utilize potato and starch dextrins as a source of carbon. The most efficient in this respect are *Bifidobacterium* and *Lactobacillus* strains, followed by *Bacteroides*, *Prevotella*, and *Clostridium* strains, irrespective of who they were isolated from. The type of potato or corn dextrins modified with different organic acids (citric, tartaric) does not affect the degree of their utilization by the studied bacteria.

4. *Bacteroides* and *Prevotella* (*Bacteroidetes*) and *Bifidobacterium* (*Actinobacteria*) isolated from the feces of overweight, obese, and normal-weight children are selectively stimulated in cultures supplemented with potato, but not corn, dextrins as the sole source of carbon.
5. The concentrations of lactic acid, SCFA, and BCFA are lower in dextrin-supplemented cultures of bacteria (irrespective of their type) isolated from the feces of overweight/obese as compared to normal-weight children; the differences are: 50%, 45%, and 64%, respectively, for media containing potato dextrin modified with citric acid as a sole carbon source; 49%, 48%, and 73%, respectively, for media containing potato dextrin modified with tartaric acid; 41%, 43%, and 74%, respectively, for media containing corn dextrin modified with citric acid; and 34%, 39%, and 54%, respectively, for media containing corn dextrin modified with tartaric acid.
6. Among the studied dextrins, potato dextrin modified with tartaric acid most intensively stimulate the growth of *Bacteroides* and *Prevotella* (*Bacteroidetes*) and *Bifidobacterium* (*Actinobacteria*), while inhibiting *Firmicutes*, and especially *Clostridium*, strains; in addition, it yields 10% more SCFA as compared to dextrin modified with citric acid.
7. The administration of low- and high-fat diets containing potato dextrin modified with citric acid to rats for 12 weeks leads to an increase and marked predominance of *Bacteroidetes* (*Bacteroides*, *Prevotella*) and *Actinobacteria* (*Bifidobacterium*) strains as over *Firmicutes*, and especially *Clostridium*, strains; furthermore, the addition of this dextrin results in a lower dietary intake (by approx. 3–6%) by rats consuming either low or high-fat diets as compared to the control groups; it also causes a 5% drop in body weight and a 12% decline in adipose tissue content in rats fed the high-fat diet as compared to controls.
8. Dextrin obtained from potato starch and modified with citric acid fed to rats for 12 weeks as a dietary component brings about favorable changes in the abundance and proportions of the major enteric bacteria: *Bacteroides*, *Prevotella* (*Bacteroidetes*), *Clostridium*, *Lactobacillus* (*Firmicutes*), and *Bifidobacterium* (*Actinobacteria*), which may be helpful in preventing overweight and obesity. These findings should be verified in clinical studies involving children.

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5. Discussion of other research accomplishments

My other research interests are associated with the following issues:

1. Chemical and physical characterization of new potato and corn dextrins resistant to enzymatic digestion.
2. Evaluation of the prebiotic properties of resistant dextrins obtained from potato starch.
3. Determination of the effects of resistant dextrins from potato starch on the gut microbiota of people in different age groups.
4. Lactose-free milk products supplemented with resistant dextrin.
5. Possibilities of using resistant starch in dietary therapy of type II diabetes mellitus.
6. Isolation and identification of bacterial strains from various natural environments.
7. The application of probiotic bacteria and bacteria isolated from children's feces in the protection and storage of fruits and vegetables.
8. The properties and applications of antimicrobial peptides.
9. The properties and antifungal applications of a new cobalt(II) coordination polymer of indole-3-carboxylic acid.

5.1. Chemical and physical characterization of new potato dextrins resistant to enzymatic digestion

An important area of my research work carried out as part of my doctoral dissertation and continued after obtaining a PhD degree was the chemical and physical characterization of new potato dextrins resistant to enzymatic digestion. That research was partially conducted under the NCBR grant project no. N N312 3261 33 led by Doctor Habil. Janusz Kapuśniak "Production and physicochemical characterization of new chemically modified and branched resistant dextrins from potato starch and their applications as prebiotics" in the years 2007–2010, in which I was an investigator. In that project, I obtained three dextrins from potato starch modified with hydrochloric acid and citric acid, and subsequently heated at 130°C for 3, 4, and 5 h. I also obtained dextrins by heating potato starch with hydrochloric acid and tartaric acid at 130°C for 2 h. For the obtained dextrins, I determined: the pH of their aqueous solutions, solubility at 37°C, the content of reducing sugars, the degree of substitution with citric acid, the distribution of molecular weights, the mean length of carbohydrate chains, and the content of the resistant fraction; I also verified whether the dextrinization process was accompanied by chemical modification. My significant accomplishment was the finding that the application of hydrochloric acid and organic acids resulted in dextrins that were readily soluble in water, had low pH, and exhibited a small degree of substitution with citric acid. The duration of heating of starch modified with citric acid (3–5 h) did not significantly affect the studied physical and chemical properties of the resulting dextrins.

Since my study design presumed that the heating of starch in the presence of citric or tartaric acid would lead to chemical modification of the resulting dextrins, I recorded the spectra of the dextrins. However, analysis of the spectra for dextrins obtained by the heating of potato starch with citric acid did not reveal chemical modification. On the other hand, the spectrum for dextrins obtained by the heating of potato starch with tartaric acid revealed a band indicating esterification. Starch depolymerization was evidenced by data obtained by means of high-performance size-exclusion chromatography (HPSEC), which was conducted in order to determine the molecular weight distribution of the obtained dextrins and to establish the weight-average molecular mass (M_W). I found the presence of three fractions for dextrins obtained in the presence of citric acid irrespective of heating time, and two fractions for dextrins produced with tartaric acid. The degree of polymerization (DP) of dextrins obtained in the presence of citric acid ranged from 22 to 30, while that for tartaric acid amounted to 11. However, of importance was not only the molecular mass of the obtained products, but also their chemical structure. To determine the number and type of branches, which is critical from the point of view of susceptibility to enzymatic digestion, I used high-performance anion-exchange chromatography (HPAEC). The mean chain length was 10.4–11.4 for dextrins

modified with citric acid and 8.2 for those modified with tartaric acid. HPAEC spectra showed that the mean chain length of all dextrins was smaller than the mean DP for the main fraction, which may indicate the presence of branches in dextrin molecules.

An important element of my research was to determine the content of the fraction resistant to enzymatic digestion in the obtained dextrins. These studies were conducted using two AOAC 2001.03 methods as well as the enzymatic-spectrophotometric method (Englyst method). The total content of dietary fiber determined by the AOAC 2001.03 method was approx. 30% for dextrins modified with citric acid and 50% for those modified with tartaric acid. However, the Englyst method (which is appropriate for resistant starch RS4 according to the Codex Alimentarius Commission) showed that the actual content of the resistant fraction in the dextrins was much higher, reaching up to 70%.

The heating of potato starch with citric and tartaric acid led to dextrins that were readily soluble in water and resistant to digestion with amylolytic enzymes, with a weight-average molecular mass that was much higher than that of commercially available preparations of resistant saccharides and with a mean chain length shorter than the mean DP of the main fraction of those dextrins, which suggested the presence of branches in the molecules. These were the fundamental findings that enabled me to expand my research interests in the area of applications of the obtained dextrins in food products.

The results of the studies constituted part of my doctoral dissertation written under the supervision Prof. Zdzisława Libudzisz and Doctor Habil. Janusz Kapuśniak, defended with honors at the Faculty of Biotechnology and Food Sciences in 2011. Prior to receiving my PhD degree, I presented those findings as a co-author of 1 monograph, 1 original work, and 2 oral presentations at international and domestic conferences. After receiving my PhD degree, I presented those findings as a co-author of 3 papers and 3 posters at international conferences and 1 oral presentation at a domestic conference.

Prior to receiving my PhD degree

Chapter in monograph:

1. Kapuśniak J., Jochym K., **Barczyńska R.**, Śliżewska K., Libudzisz Z. (2010) Enzyme resistant dextrins from potato starch as potential prebiotic, w: B. McCleary, J. M. Jones, D. Topping and J.W. van der Kamp (eds.), Dietary Fibre – New frontiers for food and health, Wageningen Academic Publishers, Netherlands, 193-215. **MNiSW = 5 pkt.**

Original paper:

1. Kapuśniak J., Jochym K., **Barczyńska R.**, Śliżewska K., Libudzisz Z. (2008) Otrzymywanie i charakterystyka opornych dekstryn ze skrobi ziemniaczanej. Zeszyty Problemowe Postępów Nauk Rolniczych, 530, 427-444. **MNiSW = 6 pkt.**

Oral presentation at international conferences:

1. Kapusniak J., Jochym K., **Barczynska R.,** Slizewska K., Libudzisz Z. (2010) Prebiotic properties of new enzyme-resistant dextrin from potato starch – from structure to functionality and health, The 10th International Hydrocolloids Conference, Szanghaj, Chiny, 20-24.06.2010. Materials pp. 65-66.

Oral presentation at domestic conference:

1. Kapuśniak J., Jochym K., **Barczyńska R.,** Śliżewska K. (2008) Otrzymywanie, charakterystyka nowych opornych, chemicznie modyfikowanych dekstryn ze skrobi ziemniaczanej, V Konferencja Naukowa „Ziemniak spożywczy i przemysłowy oraz jego przetwarzanie", Szklarska Poręba, 12-15.05.2008. Materials pp. 118-119.

After receiving my PhD degree

Original paper:

1. Kapusniak J., Kapusniak K., Ptak S., **Barczynska R.,** Żarski A. (2014) Product of thermolysis of potato starch treated witch hydrochloric and citric acids as potential prebiotic. Quality Assurance and Safety of Crops & Foods, 6 (3), 347-356. **IF_{2014} = 0.891; MNiSW = 20 pkt.**
2. Kapusniak J., Kapusniak K., Ptak S., Barczynska R., Rychter P. (2013) Products of thermal modification of starch for food and health, Proceedings of the 9th International Conference on Polysaccharides-glycoscience, Prague, Czech Republic, Nov 06-08, 2013, 5-11. **MNiSW = 15 pkt.**
3. Jochym K., Kapusniak J., **Barczynska R.**, Slizewska K. (2012) New starch preparations resistant to enzymatic digestion. Journal of the Science Food Agriculture 92 (4), 886-891. **IF_{2012} = 1.759; MNiSW = 35 pkt.**

Oral presentation at international conferences:

1. Kapusniak J., Kapusniak (Jochym) K., Ptak S., **Barczynska-Felusiak R.,** Zarski A. (2013) Products of thermal modification of potato starch for food and health, Eurofoofchem XVII, Istanbul, Turkey 07-10.05.2013. Materials pp. 235.
2. Kapusniak J., Kapusniak K., Ptak S., **Barczynska R.**, Zarski A. (2013) Products of thermal modification of starch for food and health, EPNOE 2013: "Polysaccharides and polysaccharide-derived products, from basic science to applications", Nice (France), 21-24.10.2013. Materials pp. 27.
3. Kapusniak J., Kapusniak K., Ptak S., **Barczynska R.,** Rychter P., (2013) Products of thermal modification of starch for food and health, 9th International Conference on Polysaccharides – Glycoscience, Prague (Czech Republic), 6-8.11.2013. Materials pp. 738.

Oral presentation at domestic conference:

1. Kapusniak J., Kapusniak K., Ptak S., **Barczynska R.** (2013) Produkty termicznej modyfikacji skrobi w żywieniu i dla zdrowia, XLI Sesja Naukowa Komitetu Nauk o Żywności PAN, Kraków, 2-3.07.2013. Materials pp. 66.

5.2. Evaluation of the prebiotic properties of resistant dextrins obtained from potato starch

Another important area of my research work was determination whether resistant dextrins from potato starch exhibit prebiotic properties. My research in this area are was partially carried out as part of the NCBR grant project no. N N312 3261 33 led by Doctor Habil. Janusz Kapuśniak "Production and physicochemical characterization of new chemically modified and branched resistant dextrins from potato starch and their applications as prebiotics" in the years 2007–2010, in collaboration with Doctor Habil. Katarzyna Śliżewska of the Łódź University of Technology, and constituted part of my doctoral dissertation. My studies in this respect involved pure cultures of strains with documented probiotic properties, used in food and pharmacological products (three *Lactobacillus* and two *Bifidobacterium* strains). In addition I conducted pure cultures of strains (*Escherichia coli*, *Enterococcus*, *Clostridium*, *Bacteroides*) isolated from the feces of healthy 1-year-olds, 8-year-olds, and 30-year-olds (a total of 36 strains). Furthermore, I co-cultured probiotic bacteria and strains isolated from human feces. I calculated the prebiotic index (PI) and identified and quantified products obtained from the fermentation of resistant dextrins, using HPLC. It was found that the probiotic strains and the bacteria isolated from human feces were able to utilize all the studied resistant dextrins as sources of carbon and energy. The yield of enteric bacteria (*Enterococcus*, *Clostridium*, *Bacteroides*) was lower than that of probiotic bacteria; only *Escherichia coli* strains grew as well as the probiotic ones. The bacteria isolated from the feces of adults utilized the resistant dextrins more actively than the bacteria isolated from children's feces. Among the 4 studied dextrins, the probiotic strains most efficiently utilized the dextrin modified with citric acid and heated for 4 h as well as the one modified with tartaric acid and heated for 2 h; those dextrins were the least efficiently utilized by the enteric strains.

To establish the selective effects of the studied dextrins, I conducted co-cultures of bacteria in proportions and under conditions similar to those prevailing in the colon. Based on previous research results, at this stage of research I selected two dextrins: those which were efficiently used by probiotic strains, and poorly by enteric strains (dextrin modified with citric acid, heated for 4 h, and dextrin modified with tartaric acid, heated for 2 h). Irrespective of the type of dextrin and the age of subjects from whom the isolates were derived, the environment

was beneficially dominated by the probiotic *Bifidobacterium* and *Lactobacillus* strains. However, the enteric strains (and especially *Escherichia coli*) were also capable of growth, while the cell counts of the other strains were lower. I did not find any significant differences in growth between bacteria isolated from the feces of children and those derived from adults.

I found that the determined PI values differed between the various age groups: they were the highest for 8-year-olds and the lowest for 1-year-olds. Among the two studied dextrins, the one modified with citric acid exhibited a higher PI than the one modified with tartaric acid, which indicates that the former had a greater ability to selectively stimulate the growth of *Bifidobacterium* and *Lactobacillus* strains.

As part of this wide-ranging research, I also determined the types and proportions of fermentation products obtained from the 4 studied resistant dextrins fermented by probiotic bacteria and strains isolated from human feces. The presence of resistant dextrins in the culture media did not modify the normal fermentation process either for probiotic or enteric bacteria, with the strains producing typical metabolites. Nevertheless, in the presence of dextrins modified by tartaric acid, fermentation was more homogeneous with lactic acid accounting for as much as 92% of all the products. The strains isolated from adults led to a slightly higher concentration of fermentation products. The method of producing resistant dextrins did not significantly affect the type or concentration of those products.

Prior to receiving my PhD degree, I presented those findings as an author and co-author of 3 original works, a co-author of 2 oral presentations at international conferences, an author of 1 oral presentation at a domestic conference, and as an author and co-author of 8 posters presented at international conferences and 4 at domestic conferences.

After receiving my PhD degree, I used the findings to obtain 2 patents (awarded in 2015), with the research results extensively presented in 6 papers, 2 review papers, 1 monograph, 4 oral communications (2 international and 2 domestic), and 13 posters presented at 10 international and 3 domestic conferences.

Prior to receiving my PhD degree

Original paper:

1. **Barczyńska R.**, Jochym K., Śliżewska K., Kapuśniak J., Libudzisz Z. (2010) The effect of citric acid-modified enzyme-resistant dextrin on growth and metabolism of selected strains of probiotic and other intestinal bacteria. Journal of Functional Foods, 2: 126-133. **IF_{2010} = 1,308; MNiSW = 0 pkt.**
2. Śliżewska K., **Barczyńska R.**, Kapuśniak J., Libudzisz Z. (2008), The growth and end products of *Lactobacillus* probiotics strains in the presence of resistant dextrin. Biotechnology, Serie F, Special volume 366-370. **MNiSW = 2 pkt.**

3. Kapuśniak J., **Barczyńska R.**, Śliżewska K., Libudzisz Z. (2008) Wykorzystanie opornych dekstryn przez bakterie z rodzaju *Lactobacillus*. Zeszyty Problemowe Postępów Nauk Rolniczych, 530, 445-457. **MNiSW = 6 pkt.**

Oral presentation at international conferences:

1. Kapuśniak J., Jochym K., **Barczyńska R.,** Śliżewska K. (2008) Enzyme resistant chemically modified dextrins from potato starch and their utilization by probiotic bacteria, XVI International Starch Convention Cracow-Moscow, Cracow, 16-20.06.2008. Materials pp. 85-86.
2. Kapuśniak J., Jochym K., **Barczyńska R.,** Śliżewska K., Libudzisz Z. (2009) Enzyme resistant dextrin from potato starch as potential prebiotic, 4th International Dietary Fibre Conference, Vienna, Austria, 01-03.07.2009. Materials pp. unnumbered.

Oral presentation at domestic conference:

1. **Barczyńska R.,** Śliżewska K., Kapuśniak J., Jochym K. (2008) Nowe oporne dekstryny jako potencjalne prebiotyki, „Żywność współczesna szanse i zagrożenia" XIII Sesja Sekcji Młodej Kadry Naukowej Polskiego Towarzystwa Technologów Żywności, Łódź, 28-29.05.2008. Materials pp. 93.

Posters presentation at international conferences:

1. **Barczyńska R.,** Jochym K., Kapuśniak J. (2008) Preparation, characteristics of novel enzyme-resistant dextrins from potato starch and their utilization by probiotic bacteria, Benefits and Risks of Bioactive Plant Compounds COST Action 926 Conference, Kraków Poland, 27-28.03.2008. Materials pp. 55.
2. **Barczyńska R.,** Śliżewska K., Kapuśniak J., Libudzisz Z. (2008) Fermentation products of enzyme-resistant dextrin by probiotic bacteria, European Conference on Probiotics and their Applications EUROBIO 2008, Cracow, 15-17.10.2008. Materials pp. 71.
3. Śliżewska K., Barczyńska R., Kapuśniak J., Libudzisz Z. The growth and end products of *Lactobacillus* probiotic strains in the presence of resistant dextrin, International Symposium "New Research in Biotechnology", Bucharest, 20-21.11.2008. Materials pp. 85.
4. Śliżewska K., **Barczyńska R.,** Kapuśniak J., Libudzisz Z. (2008) The growth and products of *Lactobacillus* probiotic strains the presence of resistant dextrin. Symposium "New Research in Biotechnology", Bucharest, 20-21.11.2008. Materials pp. 9.3
5. Śliżewska K., **Barczyńska R.,** Kapuśniak J., Libudzisz Z. (2009) The fermentation of new resistant dextrin by *Bifidobacterium* strains, XI Anniversary Scientific Conference 120 Years of Academic Education in Biology 45 Years Faculty of Biology "Biology – Traditions and Challenges", Sofia, Bulgaria, 27-29.05.2009. Materials pp. 168.
6. **Barczyńska R.,** Śliżewska K., Jochym K., Kapuśniak J., Libudzisz Z. (2009) The ability of *Lactobacillus casei DN-114 001*, *Bifidobacterium animalis DN-173 010* and *Clostridium* bacteria

to metabolize novel enzyme-resistant dextrins, Food and Function, International Scientific Conference on Nutraceuticals and Functional Foods, Zilina, Słowacja, 9-11.06.2009. Materials pp. 57-58.

7. **Barczyńska R.,** Śliżewska K., Kapuśniak J., Libudzisz Z. (2009) Resistant dextrin as the source of carbon for *Bifidobacterium* and *Clostridium,* Food and Function, International Scientific Conference on Nutraceuticals and Functional Foods, Zilina, Słowacja, 9-11.06.2009. Materials pp. 57.
8. Slizewska K., **Barczynska R.,** Kapuśniak J., Libudzisz Z. (2009) Resistant dextrin as the source of carbon for *Bifidobacterium* and *Bacteroides*, XXXII International Congress of the Society of Microbial Ecology and Disease, St. Petersburg, Russia, 28-29.10.2009. Materials pp. 24-25.

Posters presentation at domestic conferences:

1. **Barczyńska R.,** Śliżewska K., Kapuśniak J. (2008) Wzrost i aktywność kwasząca *Lactobacillus rhamnosus* GG w pożywce zawierającej różne źródła węgla, „Żywność współczesna szanse i zagrożenia" XIII Sesja Sekcji Młodej Kadry Naukowej Polskiego Towarzystwa Technologów Żywności, Łódź, 28-29.05.2008. Materials pp. 92.
2. **Barczyńska R.,** Śliżewska K., Kapuśniak J., Libudzisz Z. (2009) Oporne maltodekstryny jako substancje o właściwościach prebiotycznych, Interdyscyplinarne Seminarium Studenckie „Forum Młodych Nauki", Częstochowa, 22.05.2009. Materials pp. 7.
3. Jochym K., **Barczyńska R.,** Kapuśniak J., Libudzisz Z. (2009) Charakterystyka opornych dekstryn ze skrobi ziemniaczanej oraz ich wykorzystanie przez bakterie o potwierdzonych właściwościach probiotycznych, 52. Zjazd PTCh i SITPChem., Łódź, 12-16.09.2009. Materials pp. 222.
4. **Barczyńska R.,** Śliżewska K., Kapuśniak J., Libudzisz Z. (2009) Otrzymywanie nowych opornych dekstryn i ich metabolizowanie przez bakterie probiotyczne i jelitowe, Mikrobiologia w Medycynie, Przemyśle i Ochronie Środowiska, Łódź, 24-25.10.2009. Materials pp. 55-56.

After receiving my PhD degree

Patents:

1. Jochym K., Kapuśniak J., **Barczyńska R.**, Libudzisz Z. Śliżewska K. (2015) Preparat o właściwościach prebiotycznych PL.220965, Polska, Urząd Patentowy Rzeczypospolitej Polskiej
2. Kapuśniak J., Jochym K., **Barczyńska R.**, Libudzisz Z., Śliżewska K. (2015) Preparat o właściwościach prebiotycznych PL.221497, Polska, Urząd Patentowy Rzeczypospolitej Polskiej

Original paper:

1. **Barczyńska R.**, Śliżewska K., Libudzisz Z., Kapuśniak K., Kapuśniak J. (2015) Prebiotic properties of potato starch dextrins. Postępy Higieny i Medycyny Doświadczalnej 69, 1031-1041. **IF_{2015} = 0,573; MNiSW = 15 pkt.**
2. Slizewska K., **Barczynska R.,** Kapusniak J., Kapusniak K. (2015) The Effect of Tartaric Acid-modified Enzyme-resistant Dextrin from Potato Starch on Growth and Metabolism of Intestinal Bacteria, Plant Pathology & Microbiology, 6(5), 1000269. **MNiSW = 7 pkt.**
3. **Barczynska R.**, Slizewska K., Jochym K., Kapusniak J., Libudzisz Z. (2012) The tartaric acid-modified enzyme-resistant dextrin from potato starch as potential prebiotic. Journal of Functional Foods 4, 954-962. **IF_{2012} = 2,632; MNiSW = 25 pkt.**
4. Śliżewska K., Kapuśniak J., **Barczyńska R.**, Jochym K. (2012) Resistant dextrins as prebiotic. W: Carbohydrates - Comprehensive studies on giycobiology and glycotechnology. Chuan-Fa C. (red.). In Tech, Chorwacja, 2012, 261-288. **MNiSW = 5 pkt.**
5. Śliżewska K., **Barczyńska R.**, Jochym K., Kapuśniak J. (2011) Chromatograficzna analiza produktów fermentacji opornych dekstryn, Przemysł chemiczny 90(5),1045-1048. **IF_{2011}=0,414; MNiSW = 15 pkt.**
6. Śliżewska K., **Barczyńska R.**, Kapuśniak J., Jochym K. (2011) Resistant dextrins from potato starch as substances stimulating growth of *Lactobacillus* bacteria. Sepsis 1 (4), 136-137. **MNiSW = 2 pkt.**

Review paper:

1. Slizewska K., Nowak A., **Barczynska R.,** Libudzisz Z. (2013) Prebiotyki - definicja, właściwości i ich zastosowanie w przemyśle. ŻYWNOSC. NAUKA TECHNOLOGIA JAKOSC. 1(86), 5-20. **IF_{2013} = 0,311; MNiSW = 15 pkt.**
2. **Barczyńska R.**, Slizewska K., Libudzisz Z., Litwin L. (2013) Rola mikrobioty jelit w utrzymaniu prawidłowej masy ciała. Standardy Medyczne, Pediatria 1(10) 55-62. **MNiSW = 4 pkt.**
3. **Barczyńska R.** (2014) Mikrobiota jelitowa i prebiotyki - znaczenie u niemowląt i dzieci. Standardy Medyczne, Pediatria 5 (11), 711-722. **MNiSW = 4 pkt.**

Chapter in monograph:

1. Śliżewska K., Kapuśniak J., **Barczyńska R.,** Jochym K. (2012) Resistant Dextrins as Prebiotic. W: Carbohydrates - Comprehensive studies on glycobiology and glycotechnology, pod redakcją Chuan-Fa Chang, In Yech, Chorwacja, InTech Publisher, New york USA2012, ISBN 978-953-51-0864-1, s. 261-288. **MNiSW = 5 pkt.**

Oral presentation at international conferences:

1. Kapusniak J., **Barczynska R.,** Jochym K., Slizewska K., Libudzisz Z. (2011) Prebiotic properties of enzyme-resistant dextrin from potato starch. IV Congress of Polish Biotechnology and IV Eurobiotech "Four Colours of Biotechnology", Krakow Poland, 12 -15.10.2011. Materials pp. 131.

2. Kapusniak J., Jochym K., **Barczynska R**. (2012) New starch preparations with prebiotic properties – from structure to functionality and health. 5th International Dietary Fibre Conference, Rome, Italy, Centro Congressi Fontana di Trevi, 7-9.05.2012. Materials pp. 43.

Oral presentation at domestic conference:

1. Kapuśniak J., Kapuśniak K., **Barczyńska R.** (2014) Substancje prebiotyczne ze skrobi ziemniaczanej w żywieniu i dla zdrowia. Pierwsze Śląskie Seminarium Probiotyczne, Probiotyki, Synbiotyki i Prebiotyki w Profilaktyce Zdrowia. Częstochowa, 15.05. 2014. Materials pp. unnumbered.
2. Kapuśniak K., Kapuśniak J., Żarski A., Nebesny E., **Barczyńska R.** (2014) Rozpuszczalny błonnik pokarmowy ze skrobi ziemniaczanej o właściwościach prebiotycznych dla przemysłu napojów, VIII Konferencja Naukowa pt. „Ziemniak spożywczy i przemysłowy oraz jego przetwarzanie", Brunów, 12-14.05.2014. Materials pp. unnumbered.

Distinction posters presentation at international conferences:

1. Śliżewska K, Libudzisz Z., **Barczyńska R**., Kapuśniak J. (2011) Dextrine resistante avec les memes proprietes qu'un probiotigue. 39 Międzynarodowa Wystawa Wynalazków „International Exhibition of Inventions of Geneva". Genewa, Szwajcaria 6-10.04.2011 **(gold medal).**
2. Śliżewska K, Libudzisz Z., **Barczyńska R**., Kapuśniak J. (2011) Novi otporni dekstrin kao supstancija sa znaćajkama prebiotika. IV Międzynarodowe Targi Innowacji, Produktów i Technologii w Rolnictwie i Przemyśle Spożywczym „AGRO ARCA 2011". Slatina, Chorwacja 6-8.05.2011 **(gold medal).**

Posters presentation at international conferences:

1. Jochym K., **Barczynska R.,** Ptak S., Kapusniak J. (2011) Prebiotic properties of dextrins from maize starch IV Congress of Polish Biotechnology and IV Eurobiotech "Four Colours of Biotechnology", Krakow Poland, 12 -15.10. 2011. Materials pp. 132.
2. Slizewska K., **Barczynska R.,** Kapusniak J., Jochym K., (2011) The novel resistant chemically modified dextrins from potato starch, International Yakult Symposium, Vienna (Austria), 26-27.05.2011. Materials pp. 78.
3. Jochym K., Ptak S., **Barczynska R**., Kapusniak J. (2012) Enzyme-resistant dextrins from maize starch as substances with prebiotic properties, 5th International Dietary Fibre Conference Rome, Italy, Centro Congressi Fontana di Trevi, 7-9.05.2012. Materials pp. 137.
4. Slizewska K., **Barczynska R.,** Kapusniak J., Jochym K. (2012) Selectively stimulated growth of intestinal microbiota by the new enzyme-resistant dextrin, 35th International Congress of the Society for Microbial Ecology and Disease (SOMED), Valencia (Spain), 15-17.05 2012. Materials pp. 68.
5. **Barczynska R.,** Slizewska K., Kapusniak J., Kapusniak (Jochym) K., Libudzisz Z. (2013) Enzyme-resistant dextrins from potato starch stimulating growth of probiotic strains.

Microbiology and Immunology of Mucosa Probiotics Conference, Kudowa Zdrój, Poland, 28-31.05.2013. Materials pp. 58 **(poster awarded with distinction).**

6. Slizewska K., **Barczynska R.,** Kapusniak J., Jochym K. (2013) Resistant dextrins from potato starch as potential prebiotic. III Workshop on Microbiology in Health and Environmental Protection MIKROBIOT, Poland, Lodz,17-20.09.2013. Materials pp. 73.
7. Kapusniak J., Kapusniak K., **Barczynska R.** (2014) Products of termolysis of potato starch treated with hydrochloric and citric acid as potential prebiotics. International Scientific Conference on Probiotics and Prebiotics – IPC Hungary, 24–26.06.2014. Materials pp. 106-107.
8. **Barczynska R.**, Kapusniak J., Slizewska K., Litwin M., Szalecki M. (2015) Dietary fiber preparations as potential prebiotics. 2 Polish-Czech Probiotics Conference Microbiology, Immunology & allergy. Bielawa, Poland, 24-26.05.2015. Materials pp. 56.

Posters presentation at domestic conferences:

1. Ptak S., Jochym K., **Barczynska R.** (2012) Preparaty skrobiowe oporne na trawienie enzymatyczne o potencjalnych właściwościach prebiotycznych 17th Conference of young researchers section of Polish society of food technologists "Food Diversity", Kraków 10-11.05.2012. Unnumbered pages.
2. Slizewska K., **Barczynska R.,** Kapusniak J., Jochym K. (2013) Nowe oporne dekstryny ze skrobi ziemniaczanej jako potencjalne prebiotyki. III Krajowa Konferencja Naturalne substancje roślinne aspekty strukturalne I aplikacyjne, Puławy, 4-6.09.2013. Materials pp. 323-324.
3. Kapuśniak K., Żarski A., **Barczyńska R.,** Nebesny E., Kapuśniak J. (2014) Rozpuszczalny błonnik pokarmowy ze skrobi ziemniaczanej o właściwościach prebiotycznych dla przemysłu napojów. 57 Zjazd Naukowy Polskiego Towarzystwa Chemicznego i Stowarzyszenia Inżynierów i Techników Przemysłu Chemicznego. Chemia – Nadzieje i Marzenia, Częstochowa, 14-18.09.2014. Materials pp. 390.

5.3. Determination of the effects of resistant dextrins from potato starch on the gut microbiota of people in different age groups

In the years 2010–2013, as part of the NCN project (no. NN 312 335339) entitled "Modification of the gut microbiota in people of different ages under the influence of prebiotic dextrins (*in vitro* studies and *in vivo* experiments on animals)" led by Doctor Habil. Katarzyna Śliżewska of the Łódź University of Technology. As an investigator, I determined the effects of resistant dextrins on the growth of *Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Escherichia coli*, *Enterococcus*, and *Fusobacterium* strains isolated from the feces of children, 30-year-olds,

and 70-year-olds. The choice of bacteria was dictated by the fact that they are characteristic and numerically predominant in the intestines of the various age groups. I conducted *in vitro* studies in which the bacterial strains isolated from the feces of people in different age groups were cultured in media supplemented with resistant dextrins modified with citric acid as a source of carbon. It was found that resistant dextrin stimulated the growth of *Lactobacillus*, *Bifidobacterium*, *Escherichia coli*, and *Enterococcus* isolated from the feces of children, adults, and the elderly. After 24 h of culture in the presence of resistant dextrins, the largest growth was observed for *Lactobacillus* and *Bifidobacterium* strains isolated from the feces of children and adults (8.97 and 9.04 $\log_{10}$ CFU/mL, respectively), while the same strains isolated from the feces of the elderly grew very poorly and remained at the level of the inoculum. The growth of *Escherichia coli* and *Enterococcus* strains was slightly lower than that of *Lactobacillus* and *Bifidobacterium*, and amounted to 7.76–8.69 $\log_{10}$ CFU/mL and 8.30–8.82 $\log_{10}$ CFU/mL, respectively; the highest growth was observed for 1-year-olds and the lowest for 70-year-olds. *Clostridium* and *Bacteroides* strains utilized resistant dextrin as a source of carbon much less efficiently; no growth was recorded for *Clostridium* strains isolated from 1-year-old children and *Bacteroides* strains from adults and the elderly. *Clostridium* strains isolated from the feces of 8-year-olds, 30-year-olds, and the elderly grew rather poorly (from 7.3 to 7.91 $\log_{10}$ CFU/mL). As far as these studies are concerned, my major accomplishment is the finding that resistant dextrin obtained from potato starch with citric acid constitutes a source of carbon for beneficial enteric strains irrespective of the age group of the subjects from whom they were isolated; nevertheless, the studied dextrin was utilized most efficiently by the strains isolated from the feces of children and the least efficiently by those derived from the elderly.

Within the project led by Doctor Habil. Katarzyna Śliżewska I also participated in the analysis and interpretation of *in vivo* studies on rats concerning dextrin-induced changes in the gut microbiota of people in different age groups, as shown in the research reports listed below.

I presented the findings of these studies as a co-author of 1 paper and 3 posters at international conferences.

Original paper:

1. Śliżewska K., Libudzisz Z., **Barczyńska R.**, Kapuśniak J., Zduńczyk Z., Juśkiewicz J. (2015) Dietary resistant dextrins positively modulate fecal and cecal microbiota composition in young rats. Acta Biochimica Polonica 62, 677-681. **IF_{2015} = 1,187; MNiSW = 15 pkt.**

Posters presentation at international conferences:

1. Śliżewska K., **Barczyńska R.**, Kapuśniak J., Zduńczyk Z., Juśkiewicz J. (2013) Gut microbiota of rats on the consumption of resistant dextrin. The second international conference on microbial diversity „Microbial interactions in complex ecosystems". Turyn, Włochy 23-25.10.2013. Materials pp. 355-356.

2. Slizewska K., Libudzisz Z., **Barczynska R.**, Kapusniak J., Zduńczyk Z., Juśkiewicz J. (2015) The Insluence of resistant dextrins on the composition of rat's intestinal microflora (FISH metod) 2 Polish-Czech Probiotics Conference Microbiology, Immunology & allergy, Bielawa, Poland 24-26.05.2015. Materials pp. 42.
3. Slizewska K., Libudzisz Z., **Barczynska R.**, Kapusniak J., Zduńczyk Z., Juśliewicz J. (2015) Determination of the influence of the resistant dextrin on the rat's intestinal microflora. 6th International Weigl Conference on Microbiology, Gdańsk, Poland 8-10.07.2015. Materials pp. 114.

5.4. Lactose-free milk products supplemented with resistant dextrin

An important area of my research work is developing applications for the obtained resistant dextrins, with one option being their use in milk products. Due to the increasing population of individuals with lactose intolerance, I decided to determine whether resistant dextrin from potato starch modified with citric acid and heated at 130°C for 3 h, with prebiotic properties, may stimulate the growth of selected strains of enteric bacteria when incorporated in a dietary product, namely, lactose-free milk. In addition, I checked lactic acid content and the type and concentration of SCFA and BCFA, as well as the prebiotic index for the food product. This step was necessary as my previous studies into resistant dextrins were conducted under laboratory conditions on microbiological media or in fecal samples.

In these studies, my major accomplishment was the finding that potato dextrin stimulated the growth of *Lactobacillus* and *Bifidobacterium* strains, which reached 9.34 and 9.31 $\log_{10}$ CFU/mL after 12 h of incubation, making them the most abundant of the tested strains. Also after 48 h of incubation *Lactobacillus* and *Bifidobacterium* strains (9.2 and 8.92 $\log_{10}$ CFU/mL, respectively) outnumbered *Clostridium* and *Bacteroides* strains (5.83 and 5.9 $\log_{10}$ CFU/mL, respectively) by several orders of magnitude. The prebiotic index for lactose-free milk supplemented with dextrins was positive and increased with incubation time, from 0.091 after 24 h of incubation to 0.213 after 48 h. The supplementation of lactose-free milk with dextrin led to a significant increase in the concentration of lactic acid (490 mg/100 mL) and total SCFA (725 mg/100 mL), while halving total BCFA as compared to controls (lactose-free milk without dextrin).

In summary, the supplementation of food products (lactose-free milk) led to the stimulation of the growth of beneficial microorganisms, higher lactic acid and total SCFA content, and a lower concentration of BCFA, which corroborates my previous findings concerning the prebiotic properties of resistant dextrin. The intensive growth of *Lactobacillus*

and *Bifidobacterium* strains and the production of acids (and especially lactic acid) resulted in decreased pH levels in the studied milk, with the final product being a yoghurt. The consumption of lactose-free milk supplemented with resistant dextrin from potato starch may improve the gut microbiota in individuals with lactose intolerance, reduce putrefactive processes in the colon, and by the same token enhance calcium absorption from milk. However, to confirm these findings, in the future I am planning to conduct an *in vivo* investigation as well as sensory tests of the food product.

These findings were presented in 1 research paper and 1 lecture at a conference.

Original paper:

1. **Barczynska R.**, Zawierucha I., Bandurska K., Kapuśniak J. (2018) Lactose-free milk enriched with resistant dextrin. Postępy Higieny Medycyny Doświadczalnej 72, 781-787. **IF_{2017} = 0,783; MNiSW = 15 pkt.**

Oral presentation at international conferences:

1. **Barczyńska R.** (2018) Mleko bez laktozy wzbogacone dekstryną, IX Sympozjum Naukowe Probiotyki w Żywności, Kiry, 18-20 kwietnia 2018. Materials errata.

5.5. Possibilities of using resistant starch in dietary therapy of type II diabetes mellitus.

Analyzing research reports on dextrins I noticed that resistant starch could be used in the dietary therapy of diabetes. In the developed countries, several percent of the population suffer from type II diabetes mellitus involving increased resistance to insulin and impaired insulin secretion, with the incidence rate being still on the rise. Since obesity is considered a major risk factor for type II diabetes mellitus, a balanced diet may contribute to reducing the prevalence of diabetes and its complications. Indeed, owing to its hydrolytic activity, supplementation with resistant starch may play a major role in the therapy of patients with type II diabetes mellitus. The possibility to use resistant starch preparations in a diabetic diet follows directly and indirectly from the ability of these polysaccharides to normalize certain elements of carbohydrate and lipid metabolism. It has been found that in humans higher SCFA concentrations are negatively correlated with the content of free fatty acids in blood serum, thus improving the sensitivity of peripheral tissues to insulin.

The literature review on the possibilities of using resistant starch in dietary therapy of type II diabetes mellitus was presented by me as a co-author in 1 review paper.

1. Długosz E., Sklarek A., **Barczyńska R.** (2018) Skrobia oporna - perspektywy wykorzystania w dietoterapii cukrzycy typu 2 [Possibilities of using resistant starch in dietary therapy of type II diabetes mellitus]. Problemy Nauk Medycznych i Nauk o Zdrowiu 5, 5–28. **MNiSW = 5 pkt.**

5.6. Isolation and identification of bacterial strains from various natural environments

Another research interest which I pursue in parallel with the other ones is the isolation of bacterial strains from a variety of natural environments with a view to identification of strains with beneficial effects on the human body and the natural environment. I have developed my own collection of strains, which have been mostly isolated from the feces of individuals in different age groups (children, adults, and the elderly), from fermented foods, as well as from human breast milk. I have isolated a total of 300 *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Bacteroides*, *Clostridium*, *Escherichia coli*, and *Fusobacterium* strains. The strains are successively identified using API tests, FISH, and 16S RNA reactions. In the near future, the nucleotide sequences of the most beneficial strains will be deposited in the GenBank National Center of Biotechnology Information. To date, I have deposited the sequences of *Lactobacillus* isolates with an access number of MH349756.

5.7. The application of probiotic bacteria and bacteria isolated from children's feces in the protection and storage of fruits and vegetables

While chemical plant protection products are widely used to prevent and contain plant diseases, excessive use of such chemicals may lead to environmental pollution, upsetting the biological balance in agrocenoses and giving rise to new resistant plant pathogens, causing a decline in beneficial microorganisms, and contaminating food with pesticide residues which may adversely affect consumer health. As a result, researchers continue to seek new, alternative solutions that would replace, or at least complement the existing chemical-based strategies. These alternatives include substances containing effective microorganisms (EM), mostly lactic acid bacteria. My research interests also concern probiotic strains and preparations used in the protection and storage of fruits and vegetables. In my studies, I focused on the preparations I developed by co-culturing strains isolated from the feces of

1-year-olds, that is, three *Lactobacillus* and three *Bifidobacterium* strains as well as by co-culturing commercial probiotic strains (Barczyńska, 2010) isolated from food products: *Bifidobacterium bifidum Bb12*, *Bifidobacterium animalis ssp. animalis*, *Lactobacillus Shirota*, *Lactobacillus casei GG*, and *Lactobacillus casei 114 001*, as well as EmFarma™ and EmFarma Plus™ preparations made available by ProBiotics Polska™. I conducted *in vitro* studies using the disk diffusion method (Kirby–Bauer method) checking the antagonistic activity of the developed preparations against the mold *Botrytis cinerea* Pers, and then comparing their effects with those of the commercial preparations EmFarma™ and EmFarma Plus™ using the same methodology and the same culture conditions. I also conducted *in vivo* studies in which fresh strawberry fruits of the cultivar "Hokent" were sprayed with the developed preparations at 5% and 10% (OD of the undiluted preparation was 1.51), and then I compared their activity with that of the commercially available preparations EmFarma™ and EmFarma Plus™, at the same concentrations. In addition I carried out a field experiment on cucumbers ("Śremski" cultivar) and tomatoes ("Złoty Ożarowski") by spraying seedlings with the developed preparations at a concentration of 5% every 3 days for 2 months; the same experiments were also conducted using EmFarma™ and EmFarma Plus™.

The results of my *in vitro* studies enabled me to select preparations most efficiently inhibiting the development of the gray mold *Botrytis cinerea* Pers. The largest zones of inhibition of mold growth were recorded for my preparation obtained from a culture of strains isolated from the feces of children; similar results were found for the preparation EmFarma Plus™. In the field experiment, the former preparation reduced spoilage during vegetable growth and storage in both tomatoes and cucumbers (EmFarma™ had a similar effect). The treated vegetables remained fresh and firm a week longer than the untreated controls.

I presented these findings in the form of a lecture and 2 posters at domestic conferences as well as 1 paper which has been submitted to a journal and is currently being reviewed.

Original paper:

1. **Barczynska R.,** Rychter P., Herman B., Górski B. Preliminary evaluation of selected beneficial microorganisms for agricultural and postharvest application. Probiotics and Antimicrobial Proteins – under review.

Oral presentation at domestic conferences:

1. **Barczyńska R.,** Herman B., Rychter P. (2013) Wpływ preparatów probiotycznych EmFarma, EmFarma Plus i własnych na ograniczenie wzrostu i rozwoju pleśni szarej *Botritis cinerea.* II Śląska konferencja „Biologizacja rolnictwa – probiotechnologia w uprawach i hodowli. Metody zwiększające bezpieczeństwo pracy i konkurencyjność rolnictwa", Częstochowa, 13.11.2013.

Poster presentation at domestic conferences:

1. **Barczyńska R.**, Herman B., Rychter P., Dądela R. (2014) Preparaty komercyjne i własne jako środki ochrony roślin stosowane w zwalczaniu pleśni szarej *Botrytis Cinerea* Pers. 57 Zjazd Naukowy Polskiego Towarzystwa Chemicznego i Stowarzyszenia Inżynierów i Techników Przemysłu Chemicznego. Chemia – Nadzieje i Marzenia, Częstochowa, 14-18.09.2014. Materials pp. 387.
2. Rychter P., Herman B., **Barczyńska R.,** Dądela R. (2014) Zastosowanie preparatów biologicznych w przechowalnictwie warzyw. 57 Zjazd Naukowy Polskiego Towarzystwa Chemicznego i Stowarzyszenia Inżynierów i Techników Przemysłu Chemicznego. Chemia – Nadzieje i Marzenia, Częstochowa, 14-18.09.2014. Materials pp. 391.

5.8. The properties and applications of antimicrobial peptides

My additional research interest is the identification of new substances, namely peptides, with antimicrobial properties in different environments, including the human body.

For that purpose, I conducted a literature review focusing on the expression, structure, properties, and function of human cathelicidin LL-37, which may be applied as a medication against a number of bacterial and viral diseases and tumors, and in the treatment of poorly healing wounds. Cathelicidins are antibacterial peptides produced by humans and animals in response to pathogenic microorganisms. In inflammatory states, cathelicidins act directly and selectively on the cell membranes of both Gram-positive and Gram-negative pathogenic bacteria to inhibit their growth, proliferation, and colonization. Cathelicidins also inhibit and destroy bacterial biofilms, and exhibit antiviral and antiparasitic properties. They may also play a role in angiogenesis, wound healing, and apoptosis regulation. Cathelicidins may directly and selectively destroy the membranes of cancer cells without attacking normal cells, which opens up possibilities for new anticancer therapies. On the other hand, cathelicidins may also play a role in carcinogenesis as the host's defense peptide LL-37 may act as a new modulator of tumor growth and metastasis in different types of cancer. Due to its wide spectrum of activity, LL-37 could be used in pharmacotherapy. Cathelicidins may serve as a model for developing modern antimicrobial and antiviral medications. LL-37 is an excellent candidate for the design of pharmaceuticals for the treatment of infected wounds.

This literature review resulted in a co-authored paper.

1. Bandurska K., Berdowska A., **Barczyńska-Felusiak R.,** Krupa P. (2015) Unique features of human cathelicidin LL-37. BioFactors 41(5), 289–300. **IF_{2015} = 4,592; MNiSW = 35 pkt.**

5.9. The properties and antifungal applications of a new cobalt(II) coordination polymer of indole-3-carboxylic acid

In my research work, I also investigate the antibacterial and antifungal properties of newly developed chemical compounds. I have studied possible applications of a polymer of indole-3-carboxylic acid with cobalt(II) as a substance inhibiting the growth of *Aspergillus niger* and *Candida albicans*. The new cobalt(II) complex $[Co(I3CAH)_2(H_2O)]_n$, in which *n* is the number of $[Co(I3CAH)_2(H_2O)]$ units in the coordination polymer, and I3CAH is an O-deprotonated ion of indole-3-carboxylic acid ($I3CAH_2$), was synthesized and characterized using single-crystal X-ray diffraction and infrared spectroscopy. In the future, this polymer may be used for the protection of plants and food from molds (to replace toxic compounds such as biphenyl E230, ortho-phenylphenol E231, and sodium orthophenyl phenol E232) or for the production of antifungal medication.

I used the plate method, whereby I plated the studied strains on PDA medium and added cobalt(II) coordination polymer of indole-3-carboxylic acid to study the effects of this compound on the growth of *Aspergillus niger* and *Candida albicans*. After two days of experiment, the cell count of *Candida albicans* was 3.2×10^2 CFU/mL as compared to 5.6×10^8 CFU/mL for control samples without the polymer, which indicates the high effectiveness of the studied compound. The new polymer also significantly reduced the growth of *Aspergillus niger*, with an inhibition zone of 4.0–5.1 cm after 2 days (at a plate diameter of 5.5 cm). However, after 7 and 14 days of incubation, the inhibition zone shrank to approx. 2.8 cm (14 days).

The studies led to a patent application and 1 research paper.

Patent Application:

1. Związek kompleksowy kobaltu(II), oraz jego zastosowanie Nr zgłoszenia P.425954, 16.06.2018r.

Original paper:

1. Szmigiel K., Nentwig M., Oeckler O., **Barczyńska-Felusiak R.,** Morzyk-Ociepa B. (2018) Crystal structure, vibrational spectroscopic characterization and antifungal activity of a novel coordination polymer of indole-3-carboxylic acid with cobalt(II) and a comparison with the isostructural Zn(II) complex. Inorganic Chemistry Communications 97, 56-62. **IF_{2017} = 1,810; MNiSW = 25 pkt.**

This invention was awarded with a silver medal at the International Warsaw Invention Show IWIS 2018.

My experience in microbiology, gut microbiota, probiotics, resistant starch, and dextrins has been appreciated internationally in that I have been invited to review papers in these fields by prestigious journals, such as The Journal of Nutrition (4.398), Beneficial Microbes (IF=2.923), Journal of the Science of Food and Agriculture (IF=2.463), Plant Foods for Human Nutrition (IF=2.368), PLOS ONE (IF=2.806), Journal of Functional Foods (IF=3.144), Folia Microbiologica (IF=1.521), Journal of Medicinal Food (IF=1.954), Quality Assurance and Safety of Crops & Foods (IF=0.558). I have reviewed a total of 31 papers.

Table 1. Summary of published papers with MNiSW points and Impact Factor scores

L.p	Publication	Number of publications		MNiSW points	*Impact Factor* by year	Sumary MNiSW points
		Before PhD degree	After PhD degree			
A. Publications in scientific journals with an Impact Factor (IF), in the Journal Citation Reports (JCR) database						
1.	Polish Journal of Microbiology 2015		1	15	0,697	15
2.	Polish Journal of Microbiology 2018		1	15	0,784	15
3.	Plant Foods for Human Nutrition		1	35	2,368	35
4.	Journal of Functional Foods 2015		1	45	3,574	45
5.	Journal of Functional Foods 2016		1	45	3,144	45
6.	Journal of Functional Foods 2010	1		0	1,308	0
7.	Journal of Functional Foods 2012		1	25	2,632	25
8.	Acta Biochimica Polonica		2	15	2x1,187	2x15
9.	Quality Assurance and Safety of Crops & Foods		1	20	0,891	20
10.	Journal of the Science Food Agriculture		1	35	1,759	35
11.	Postępy Higieny i Medycyny Doświadczalnej 2015		1	15	0,573	15
12.	Postępy Higieny i Medycyny Doświadczalnej 2018		1	15	0,783	15
13.	Przemysł chemiczny		1	15	0,414	15
14.	ŻYWNOSC. NAUKA TECHNOLOGIA JAKOSC.		1	15	0,311	15
15.	BioFactors		1	35	4,592	35
16.	Inorganic Chemistry Communications		1	25	1,810	25
Summary		**1**	**16**		**28,014**	**385**
B. Scientific publications in journals that do not have an IF impact factor listed in Part B of the Minister's list						
1.	Zeszyty Problemowe Postępów Nauk Rolniczych	2		6		2x6
2.	Standardy Medyczne, Pediatria		2	4		2x4
Summary		**2**	**2**			**20**

C. Chapters in scientific monographs in English						
1.	Carbohydrates - Comprehensive studies on glycobiology and glycotechnology		1	5		5
2.	Dietary Fibre – New frontiers for food and health, Wageningen Academic Publishers, Netherlands	1		5		5
Summary		**1**	**1**			**10**
D. Other						
1.	Journal of Nutritional Ecology and Food Research		1	0		0
2.	Plant Pathology & Microbiology		1	7		7
3.	Problemy Nauk Medycznych i Nauk o Zdrowiu		1	5		5
4.	Biotechnology, Serie F	1		2		2
5.	Sepsis		1	2		2
6	Proceedings of the 9th International Conference on Polysaccharides-glycoscience, Prague, Czech Republic		1	15		15
Summary		**1**	**5**			**31**
Summary of all publications		**5**	**24**		**28,04**	**446**
E. Patent applications						
1.	Związek kompleksowy kobaltu (II), oraz jego zastosowanie Nr zgłoszenia P.425954, 16.06.2018r.		1	2		2
Summary		**0**	**1**			**2**
F. Patents						
1.	Preparat o właściwościach prebiotycznych PL.220965, 18.06.2015		1	30		
2.	Preparat o właściwościach prebiotycznych PL.221497, 18.06.2015		1	30		
Summary		**0**	**2**			**60**

17th December, 2018