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Fermentation of Commercial Resistant Starch Products by Lactic Acid Bacteria Isolated from Various Foods

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HIGHLIGHTS

- *L. plantarum* (K2-1) is the best strain utilizing Type-2 resistant starch.

- *L. sakei* (8.P1.8) is the best strain utilizing Type-3 resistant starch.

- *L. sakei* (5.P1.5) is the best strain utilizing Type-4 resistant starch.

- *L. sakei* bacterial group used all resistant starch products at the best level.

Abstract: This study was aimed to evaluate the capacity of lactic acid bacteria to use commercial resistant starch types as prebiotics. In addition, the prebiotic capacities of resistant starch types were compared. Among the commercially resistant starch types, type 2 (Hi-Maize 260), type 3 (Novelose 330), and type 4 (Demirpolat) resistant starches were used. Lactic acid bacteria have been isolated from kefir, pastırma, cucurbita and beetroots pickled. Four different lactic acid bacteria were purchased as type cultures. It was revealed that Hi-maize 260, Novelose 330 and Demirpolat commercial resistant starch products were fermented by all lactic acid bacteria in the study. At the end of the study, the highest bacterial density was determined in *L. sakei* (8.P1.8) utilizing Novelose 330, in *L. plantarum* (K2-1) using Hi-maize 260 and in *L. sakei* (5.P1.5) using Demirpolat at the 48th hour. In general, it was found that the *L. sakei* bacterial group used all resistant starch products at the best level at the 48th hour. Commercial-resistant starch products are used as carbon sources by lactic acid bacteria. Therefore, these resistant starch products can be accepted as a prebiotic component and contribute to the development of new functional foods in the food industry.

Keywords: fermentation; lactic acid bacteria; resistant starch; prebiotics; probiotics.

INTRODUCTION

Lactic acid bacteria (LAB), one of the most important microorganism groups for the food industry, includes a large heterogeneous group of gram-positive, catalase-negative, acid-tolerant, non-spore-forming cocci or rod bacteria that produce lactic acid as a final product through the fermentation of carbohydrates. Depending on the fermentation product, LAB is classified as obligate homofermentative bacteria that produce lactic acid (about 90%) as the main product, or obligate heterofermentative bacteria that produce various

metabolites, including lactic acid, acetic acid, ethanol, and carbon dioxide [1]. The most well-known genus of the LAB is Lactobacillus. This genus contains many species. So far, 262 species have been classified in the genus Lactobacillus. In recent years, the genus Lactobacillus has been reclassified by scientists and divided into 25 genera which consist of Lactobacillus, Paralactobacillus and 23 new genera [2].

Lactic acid bacteria are widespread in nature. They are found in various foods (fermented dairy products, meat, vegetable etc.), soil, water and in gastrointestinal and urogenital tracts of humans and animals [3, 4]. Because of their ambidextrous metabolism and ability to synthesize a wide variety of beneficial metabolites in addition to lactic acid production, they are widely used in biotechnology applications and the food industry [4]. In the food industry, they are mainly used in the fermentation of many foods [3]. During the fermentation, LAB produces organic acids and other metabolites that increase flavour development in food, prevent spoilage and have beneficial properties. Especially in the dairy industry, they are used as starter cultures. In food preservation, bacteriocins produced by LAB have an antimicrobial effect against pathogens and microorganisms that cause spoilage [3, 5]. In the food industry, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Weisella, Carnobacterium, Oenococcus, Tetragenococcus, Aerococcus, Vagococcus and Pediococcus genera, especially Lactobacillus genera are used functionally and technologically [1].

Lactic acid bacteria have many functional properties such as enriching sensory qualities, increasing nutrient density, and increasing the bioavailability of nutrients. Also, they are one of the most widely used bacterial groups as probiotics [1]. Although accepted definitions for probiotics have been made by various researchers, a probiotic microorganism was defined by the FAO / WHO in 2001 as a "living microorganism that has positive effects on the health of the host when consumed in sufficient quantities" [6]. In 2014, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined probiotics as preserving the definition made by the FAO/WHO after making some minor grammatical corrections [7]. Probiotics are often added to foods as supplements. Probiotics provide benefits such as modulating the gut microbiota, lowering cholesterol levels, and regulating inflammatory and immune responses [8]. Understanding the importance of gut microbiota on human health and with the use of probiotics to modulate the microbiota, the number of studies on this issue has increased, different types of microorganisms have been discovered with the development of scientific analysis methods, and with the use of probiotics in clinical applications, a wide variety of gut-related or directly observable effects of probiotics have been discovered.

Another term that comes up with the concept of probiotics is prebiotics. Prebiotics, which are famous for supporting the development of probiotics, are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" [9, 10]. Today, various nutrients are being investigated in terms of their prebiotic capacity. It has recently been demonstrated that resistant starch types have potential prebiotic capacity.

Resistant starch is defined as "the sum of starch and starch products that are not absorbed and degraded in the small intestine of healthy individuals". Resistant starch is not digested in the small intestine. It is fermented in the colon [11]. When resistant starch reaches the colon, it is fermented by beneficial bacteria in the intestinal microbiota and then it makes the production of short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate [12]. Therefore, resistant starch contributes to the modulation of the intestinal microbiota and promises potential as a prebiotic component due to this function [13].

Resistant starch is divided into five subtypes depending on its structure. Type 1 resistant starch is the physically preserved starch. Type 2 resistant starch is the B-type crystalline ungelatinized resistant starch granules. Type 3 resistant starch is retrograde starch. Type 4 resistant starch is the chemically modified starch produced by cross-linking with chemical reagents. Type 5 resistant starch is the starch consisting of amylose-lipid complexes [14].

In a limited number of studies, it has been demonstrated that resistant starch can be converted into short-chain fatty acids after fermented by Bifidobacterium and Lactobacillus species which first come to mind as probiotic microorganisms in the microbiota and that it positively affects the development of these microbial groups [15]. In recent years, the number of studies in which the prebiotic capacities of resistant starch types are investigated has increased. However, the number of studies in which the prebiotic capacities of commercially produced resistant starch types are compared is very limited. The present study was carried out to investigate the capacity of LAB isolated from various foods to utilize commercially produced resistant starch types as carbon sources and to compare the prebiotic capacities of resistant starch types.

MATERIAL AND METHODS

Microorganisms

In this study, 22 LAB strains provided by the Department of Biology, Faculty of Science of Anadolu University and 4 different types of cultures were used. Type cultures were purchased from the American Type Culture Collection (ATCC), Manassas, Virginia, USA. More detailed information about the used strains is given in Table 1. All microorganisms were stored at -80°C in 20% glycerol (v/v) and were pre-cultivated twice in Man, Rogosa and Sharpe (MRS) broth at 30 °C for 18 h before they were used.

Table 1. Bacteria Strains and Sources

Strains from our laboratory collection			
Strains	**Identification**	**Source**	**References**
K2-1	*Lactiplantibacillus plantarum*	Kefir	
K2-3	*Lactiplantibacillus plantarum*	Kefir	
K2-4	*Lactiplantibacillus plantarum*	Kefir	
K2-5	*Lactiplantibacillus plantarum*	Kefir	16
K2-6	*Lactiplantibacillus plantarum*	Kefir	
K2-7	*Lactiplantibacillus plantarum*	Kefir	
K2-22	*Lactiplantibacillus plantarum*	Kefir	
KM4-3	*Lactiplantibacillus plantarum*	Kefir	
K2-19	*Levilactobacillus brevis*	Kefir	
K2-20	*Levilactobacillus brevis*	Kefir	
K2-21	*Levilactobacillus brevis*	Kefir	16
KM1-4	*Levilactobacillus brevis*	Kefir	
KM2-8	*Levilactobacillus brevis*	Kefir	
K2-2	*Lacticaseibacillus paracasei*	Kefir	
K2-10	*Lacticaseibacillus paracasei*	Kefir	16
KM 5	*Lacticaseibacillus paracasei*	Kefir	
5.P1.5	*Latilactobacillus sakei*	Pastırma	
8.P1.8	*Latilactobacillus sakei*	Pastırma	17
28.P2.5	*Latilactobacillus sakei*	Pastırma	
A5	*Latilactobacillus curvatus*	Pickled cucurbita	
P3X	*Latilactobacillus curvatus*	Pickled beetroots	18
P5	*Latilactobacillus curvatus*	Pickled beetroots	
Type cultures			
Lacticaseibacillus rhamnosus GG ATCC 53103 (LGG)			Commercially available
Lacticaseibacillus rhamnosus (Hansen) ATCC 9595			Commercially available
Lacticaseibacillus casei ATCC 393			Commercially available
Lactiplantibacillus plantarum subsp. plantarum ATCC 8014			Commercially available

LAB strains were previously isolated and identified from kefir, pastırma, pickled cucurbita and pickled beetroots. Kefir is a fermented milk beverage traditionally consumed in Turkey. It is prepared by inoculating cow, sheep, or goat milk with kefir grains for a day or two at room temperature. In this study, bacteria isolated from ten different kefir samples were used [16]. Pastırma is an uncooked meat product made from whole beef or buffalo muscles and coated with fenugreek. Pastırma is produced because of fermentation involving salting and drying processes. Pastırma production takes about a month, depending on the muscle size used. In this study, bacteria isolated from ten different pastırma samples were used [17]. Pickles are a product that gains durability because of the protective effect of lactic acid and salt in the environment, which is formed by the fermentation of fruits and vegetables in brine containing certain concentrations of salt or in their own juices by lactic acid bacteria. Bacteria isolated from two different pickle samples were used in this study [18]. *L. plantarum* (n=8), *L. brevis* (n=5) and *L. paracasei* (n=3) strains were isolated from kefir samples [16]. *L. sakei* (n=3) and *L. curvatus* (n=3) strains were isolated from pastırma [17] and pickle samples [18],

respectively. Carbohydrate fermentation test of the LAB strains were performed using an API 50CHL identification kit (Bio-Merieux, France). Strains were identified by automated EcoRI ribotyping. EcoRI ribotyping was performed with an Automatics RiboPrinter® Microbial Characterization System (Qualicon Inc., Wilmington, DE) and the EcoRI DNA preparation kit as described in the manufacturer's operations and analytical guides. The ribotype profiles of the isolates were compared with the reference DuPont identification database [16-18].

Materials

Type 2 (Hi-maize 260) and type 3 (Novelose 330) resistant starch were obtained from Ingredion Incorporated, Westchester, Illinois, USA. Type 4 resistant starch was obtained from Demirpolat Ltd, Konya, Turkey. More detailed information about the used resistant starches is given in Table 2. MRS broth, Buffered Peptone Water (BPW) and all other chemicals were purchased from Merck, Turkey.

Table 2. Properties of Resistant Starch

Name of Resistant Starch		Carbohydrate (g)	Protein (g)	Fat (g)	Dietary Fiber (g)	Resistant Starch (%)	Moisture content (%)
Type II	Hi-maize 260	40.6	0.6	0.8	48.0	53.0	10.0-14.0
Type III	Novelose 330	87.8	0.5	1.0	23.0	30.0	8.0
Type IV	Demirpolat	0	0.4	0.1	86.36	86.36	13.14

Determination of Prebiotic Activity

The prebiotic activity of resistant starches was evaluated the according to the method proposed by Wang and coauthors [19] with some modifications. Studies were carried out simultaneously for all resistant starch types. After the carbon-free MRS broth was prepared, resistant starch (20 gr/L) was added to the medium instead of glucose. Three different modified MRS broths containing Type 2 RS, Type 3 RS and Type 4 RS as carbon sources were prepared. Glucose-added MRS broth and carbon-free MRS broth were used as a positive and negative control, respectively. All mediums were sterilized by autoclaving. For the study after the strains were pre-cultivated twice in MRS broth, optical densities of 18-hour second active cultures of strains were adjusted 0.6 ± 0.002 at 600 nm. 1% (v/v) volume sample was taken from here and inoculated into each prepared medium. Then, tubes were incubated at 30 °C for 72 h. Viable cell count was performed in MRS agar plates with plate counting method after 48th and 72nd h of incubation. For the enumeration, a 10x serial dilution of each sample was prepared in BPW and 100 µL of each dilution was spread onto an MRS agar plate. After the 24-48 h incubation at 30 °C plates with 30–300 colonies were counted. To determine the number of colonies in 1 ml (CFU mL-1), the number of colonies in the counted petri dish was multiplied by the total dilution factor, and then divided by the volume (mL) transferred to the counted Petri dish. All the assays were performed twice and the average of two repetitions was used for calculations. The results were expressed as log CFU mL-1.

Statistical Analysis

The statistical analysis of the data obtained from the study was performed on the computer by using the IBM SPSS Statistics V.23.0. Whether the data were normally distributed was checked with the Shapiro-Wilk test. Normally distributed data were given as $\bar{x} \pm SD$, and non-normally distributed data were given as median. The ANOVA was used for the comparison of more than two groups. To determine which group was different from the others, the Bonferroni test was used for those meeting the homogeneity assumption, and Tamhane's T2 test was used for those not meeting the homogeneity assumption. p values less than 0.05 were considered statistically significant.

RESULTS

In the present study, the prebiotic capacities of 3 different commercially resistant starch products (Hi-maize 260, Novelose 330 and Demirpolat) were investigated using 26 LAB from 7 different species. Glucose was used as a control parameter. The resistant starch content used differs from each other. While the highest amount of resistant starch is in Demirpolat resistant starch, the lowest amount is in Novelose 330. The difference between the amounts of resistant starch in this type of product affects the capacity of LAB to use them as a prebiotic. In the literature, it was observed that the time needed to use resistant starch types as prebiotics by LAB was more than that needed by glucose and that the number of viable bacteria was calculated at the 48th and 72nd hours [20-22]. Therefore, in the present study, viable cell counts were

determined at the 48th and 72nd hours of incubation. In addition, the capacity of the bacterial groups to utilize commercial resistant starch types and the prebiotic capacities of the bacterial groups were compared statistically. In Table 3, the viable cell count of LAB strains to utilize glucose and commercial-resistant starch types as carbon sources were given.

The analysis of the resistant starch utilization capacity of LAB at the 48th hour demonstrated that in some bacterial species, values were close to those of glucose. Moreover, in some bacterial species, commercial-resistant starches have been found to have higher bacterial density than glucose. At the end of the study, the highest bacterial density was determined in *L. sakei* (8.P1.8) utilizing Novelose 330, in *L. plantarum* (K2-1) using Hi-maize 260 and in *L. sakei* (5.P1.5) using Demirpolat at the 48th hour. The glucose utilization capacity of LAB is high at the 48th hour, but at the 72nd hour, the bacterial density tends to decrease in general. However, it was determined that some LAB concentrations in commercial-resistant starch types increased at the 72nd hour. This increase did not occur in all commercial-resistant starch types in the same bacteria, which can be explained by the fact that commercial-resistant starch types are different and that their resistant starch contents vary. A general evaluation of our data has indicated that LAB can utilize Hi-maize 260, Novelose 330 and Demirpolat commercial-resistant starch types as prebiotics.

In the present study, LAB was also grouped based on species and their capacity to use resistant starch types was compared. Commercially purchased type cultures were accepted as the control group, and the other bacteria were divided into groups based on species (*L. plantarum, L. brevis, L. paracasei, L. sakei and L. curvatus*). As expected, it was observed that all LAB groups used glucose and there was no significant difference between the groups at the 48th hour. The one-way ANOVA analysis of the utilization of commercial-resistant starch products by LAB strains revealed a significant difference between the three resistant starch types (Table 4). In addition, the same analysis revealed that there is a significant difference between different LAB species in terms of the capacity to use the same resistant starch type (Table 4). In general, it was determined that the *L. sakei* bacterial group used all resistant starch products at the best level at the 48th hour. After the post hoc tests, it was determined that the statistical difference was between *L. paracasei* and *L. sakei* in the Hi-maize resistant starch. For Novelose 330, there was a significant difference between *L. sakei* and control groups, *L. brevis* and *L. curvatus* groups. In post hoc analysis, it was determined that there was no significant difference between Demirpolat-resistant starch product and bacterial groups. The comparison of the groups using the Hi-maize and Novelose 330 resistant starch at the 48th hour demonstrated that the lowest value belonged to the *L. paracasei* group and the highest value belonged to the *L. sakei* group.

According to the one-way ANOVA analysis of the bacterial density at the 72nd hour, a significant difference was determined among the LAB species in terms of the capacity to use both glucose and Demirpolat-resistant starch. In general, it was determined that the *L. sakei* bacterial group used Hi-maize 260, *L. curvatus* bacterial group used Novelose 330 and Demirpolat at the best level at the 72nd hour. As a result of the ANOVA analysis in this study, it was revealed that LAB species use resistant starch as a prebiotic. Post-hoc analysis was performed to determine which LAB provided this result. After the post hoc tests, it was revealed that the statistical difference was between *L. plantarum* and *L. sakei* when glucose was used. For Demirpolat, there was a significant difference between the control and *L. curvatus* bacteria groups. Of the bacterial groups using glucose, at the 72nd hour, *L. sakei* had the highest value, and *L. plantarum* had the lowest value. Of the bacterial groups using Demirpolat-resistant starch, *L. curvatus* had the highest value, and *L. paracasei* had the lowest value at the 72nd hour.

Table 3. Viable cell counts (log10 CFU mL-1) of lactic acid bacteria in commercially produced resistant starch fermentation at 48th and 72nd hours

Bacteria Strains		Glucose 48 h	Hi-maize260 48 h	Novelose330 48 h	Demirpolat 48 h	Glucose 72 h	Hi-maize260 72 h	Novelose330 72 h	Demirpolat 72 h
L. rhamnosus ATCC 53103		8.26±0.07	7.47±0.41	7.73±0.05	7.78±0.13	7.78±0.03	7.40±0.22	7.70±0.05	7.88±0.40
L. rhamnosus ATCC 9595		7.96±0.17	8.15±0.09	8.27±0.06	8.13±0.04	7.97±0.08	8.03±0.07	8.03±0.07	7.96±0.06
L. casei ATCC 393		8.99±0.17	8.18±0.01	8.20±0.08	8.03±0.08	8.87±0.17	7.79±0.01	8.04±0.13	8.03±0.19
L. plantarum ATCC 8014		6.71±0.22	8.28±0.13	8.27±0.15	8.19±0.36	7.52±0.04	7.57±0.12	7.69±0.43	7.93±0.07
1	K2-1	7.89±0.03	9.16±0.01	8.34±0.08	7.92±0.05	7.72±0.08	8.37±0.01	8.23±0.12	8.36±0.16
	K2-3	7.90±0.12	8.21±0.49	8.05±0.04	8.19±0.06	7.69±0.08	8.07±0.27	8.02±0.08	7.98±0.17
	K2-4	8.50±0.01	8.34±0.07	8.36±0.29	8.68±0.49	7.51±0.06	8.67±0.06	8.60±0.37	8.82±0.07
	K2-5	8.29±0.01	8.48±0.07	8.52±0.10	8.50±0.11	7.46±0.04	8.45±0.12	8.59±0.04	8.53±0.18
	K2-6	8.94±0.04	8.04±0.07	8.28±0.31	8.27±0.04	6.33±0.14	6.94±0.18	8.04±0.33	8.02±0.03
	K2-7	7.84±0.11	8.19±0.21	8.33±0.07	8.07±0.04	7.64±0.07	8.15±0.00	8.38±0.20	7.97±0.03
	K2-22	7.98±0.01	7.78±0.09	8.17±0.01	7.95±0.04	7.35±0.09	7.58±0.08	7.60±0.39	7.83±0.06
	KM4-3	7.97±0.04	7.85±0.04	8.15±0.06	7.83±0.04	7.38±0.07	7.79±0.10	8.23±0.09	7.94±0.06
	K2-19	7.93±0.01	7.76±0.06	7.80±0.20	8.20±0.10	7.38±0.07	8.12±0.08	8.42±0.10	8.26±0.11
	K2-20	7.91±0.05	8.10±0.11	8.15±0.03	8.06±0.09	7.68±0.01	7.89±0.03	8.20±0.03	8.13±0.09
2	K2-21	7.70±0.03	7.63±0.10	7.79±0.10	7.65±0.05	7.26±0.07	7.46±0.40	7.79±0.22	7.80±0.08
	KM1-4	7.80±0.11	7.88±0.07	7.81±0.17	7.23±0.07	7.12±0.25	7.62±0.08	7.74±0.06	7.50±0.02
	KM2-8	8.26±0.06	8.30±0.08	8.70±0.11	8.36±0.05	8.60±0.19	8.29±0.10	8.95±0.04	8.35±0.02
	K2-2	8.90±0.18	8.04±0.00	7.92±0.37	7.07±0.24	8.55±0.16	7.64±0.38	8.56±0.09	7.60±0.09
3	K2-10	8.33±0.05	7.05±0.02	7.30±0.19	6.93±0.33	8.15±0.07	6.82±0.02	7.03±0.28	7.45±0.96
	KM 5	8.20±0.13	7.85±0.13	8.40±0.04	8.13±0.06	8.60±0.08	8.11±0.07	8.52±0.03	8.09±0.06
	5.P1.5	9.04±0.04	8.88±0.16	9.01±0.07	8.92±0.30	8.63±0.016	8.58±0.07	8.07±0.29	8.19±0.07
4	8.P1.8	8.76±0.30	8.80±0.08	9.07±0.30	8.57±0.05	9.17±0.09	8.25±0.12	8.76±0.39	8.43±0.57
	28.P2.5	8.73±0.09	8.71±0.12	8.79±0.13	8.56±0.30	8.36±0.19	8.76±0.44	8.42±0.45	8.34±0.22
	A5	8.78±0.04	8.36±0.01	8.72±0.02	8.44±0.06	8.74±0.02	8.53±0.12	8.53±0.12	8.50±0.03
5	P3X	8.67±0.03	8.56±0.06	8.47±0.12	8.54±0.11	8.49±0.07	8.36±0.02	8.48±0.31	8.49±0.06
	P5	8.24±0.21	8.08±0.01	8.08±0.15	8.08±0.06	7.33±0.14	8.01±0.09	8.48±0.11	8.40±0.04

[1] *L. plantarum,* [2] *L. brevis,* [3] *L. paracasei,* [4] *L. sakei,* [5] *L. curvatus*

Table 4. Relationship between resistant starch fermentation of bacterial groups at 48th and 72nd hours

	Glucose[1] 48 h mean ± SD		Hi-maize 260[1] 48 h mean ± SD		Novelose 330[1] 48 h mean ± SD		Demirpolat[2] 48 h mean ± SD		Glucose[1] 72 h mean ± SD		Hi-maize 260[1] 72 h mean ± SD		Novelose 330[2] 72 h mean ± SD		Demirpolat[2] 72 h mean ± SD	
Control	7.97±0.94		8.01±0.37		8.11±0.26		8.03±0.17		8.03±0.58		7.69±0.27		7.86±0.19		7.95±0.06	
L. plantarum	8.16±0.38		8.25±0.43		8.27±0.14		8.17±0.29		7.38±0.44		8.00±0.55		8.21±0.32		8.18±0.34	
L. brevis	7.92±0.21		7.93±0.26		8.04±0.39		7.89±0.45		7.60±0.58		7.87±0.34		8.21±0.49		8.00±0.35	
L. paracasei	8.47±0.37	p value 0.098	7.64±0.52	p value 0.016**	7.87±0.55	p value 0.004*	7.37±0.65	p value 0.006*	8.43±0.24	p value 0.009*	7.52±0.65	p value 0.092	8.03±0.87	p value 0.422	7.71±0.33	p value 0.040**
L. sakei	8.84±0.17		8.79±0.08		8.95±0.14		8.68±0.20		8.71±0.41		8.53±0.26		8.41±0.34		8.31±0.12	
L. curvatus	8.56±0.28		8.33±0.23		8.42±0.32		8.35±0.23		8.18±0.75		8.30±0.26		8.49±0.02		8.46±0.05	

[1] Bonferronni test; [2] Tamhane T2 test, * p<0.01, ** p<0.05

DISCUSSION

In the current study, besides the use of Hi-maize 260 and Novelose 330 commercial resistant starches (which are evaluated in other studies in the literature), the prebiotic capacity of Demirpolat type 4 resistant starch, which was produced in Turkey, was also investigated for the first time. In the present study in which different species of LAB were included, both cultures isolated from various foods and commercially available type cultures were used. Our current review of the literature demonstrated that only in one study, utilization capacity as the prebiotic of different types of commercially resistant starches by probiotic bacteria was examined. In that study, type 2 (S4180, Hi-maize 260, Hi-maize 958), type 3 (Novelose 330), type 4 (Versafibe 1490, Versafibe 2470) resistant starches and *Bifidobacterium* type cultures were included. According to the results of that study, *Bifidobacterium* type cultures utilized resistant starches at different rates [23]. In our study, all the LAB species utilized commercial-resistant starch types like the way they utilized the control group.

In several publications in the literature, the capacity of lactic acid bacterial species to utilize resistant starch types as prebiotics was investigated; however, the results obtained differed from one study to another [22, 24-26]. In a study involving *Lactobacillus acidophilus FTCC 0291*, *L. casei FTCC 0442*, *Lactobacillus bulgaricus FTCC 0411* bacteria, it was revealed that type 2 resistant starches (Hi-maize 240 and Hi-maize 1043) could be utilized as a prebiotic source [27]. Whereas in another study, it was determined that *L. brevis ATCC 14869* type culture did not utilize S4180 type 2 resistant starch as a carbon source [28].

When similar studies using the type cultures included in our study were screened in the existing literature, it was seen that resistant starches isolated from foods were used by some type cultures and the results of the studies varied. A study in which *L. casei ATCC 393* and *L. rhamnosus ATCC 53103* type cultures were included, reported that these cultures did not ferment resistant starch isolated from fava bean [29]. In another study in which the *L. plantarum ATCC 8014* strain was used, the prebiotic activity score of breadfruit-resistant starch and flour was investigated. However, *L. plantarum* did not utilize breadfruit flour as a carbon source and the prebiotic activity score of breadfruit-resistant starch was lower than glucose [21]. On the other hand, in various studies in which *L. casei ATCC 393* and *L. rhamnosus GG* strains were used, it was determined that these bacteria utilized resistant starches isolated from foods as prebiotics [30, 31]. It has been shown that *L. plantarum 61-1* and *Lactobacillus delbrueckii 42-1* strains isolated from traditional fermented milk products and *Lactobacillus rhamnosus strain GG* (ATCC 53103) purchased as type cultures can ferment commercial type 3 resistant starch (Shanghai Yuanye Biotechnology Co., Ltd. Shanghai, China) [32]. The fermentation of resistant starches obtained from various kinds of rice with type cultures of *L. brevis* (MTCC 01), *L. plantarum* (MTCC 021), and *L. casei* (MTCC 297) was investigated. It was observed that lactic acid bacteria fermented rice-resistant starches [33]. The fermentation of isolated resistant starch nanoparticles by the *Lactiplantibacillus plantarum* strain was investigated. In the study, it was found that the number of viable bacterial cells and butyric acid concentration were higher in the bacteria group to which resistant starch was added [19]. Fermentation of four different types of commercial type 4 resistant starches (Versafibe 1490, Versafibe 2470, Versafibe 2480 and Novelose 3490) in vivo in a rat model was investigated. It was revealed that all resistant starch types can be fermented in the intestine, and cecal wet weight and cecal propionate content were significantly higher in the resistant starch groups compared to the control group [34]. It is known that LAB strains utilize oligosaccharides. However, members of LAB species show a strain-dependent manner. Endo and coauthors [35] reported that 2 of the 3 *L. paracasei* strains used oligosaccharides like 1-kestose, nystose, and fructooligosaccharide as prebiotics. In the same study, the potential of using of oligosaccharides by 2 different *L. sakei* strains were also investigated and it was observed that *L. sakei* strains could metabolize galactooligosaccharides and Xylo oligosaccharides more than raffinose and lactosucrose. Kim and coauthors [36] reported that *L. curvatus* strain isolated from Korean traditional fermented seafood the growth rate using isomaltose-oligosaccharides was significantly higher than the growth rate of the furctooligosacharides and inulin.

The present study is the first study in the literature to compare the use of different commercially resistant starch products as a carbon source by different LAB species. As far as we know, *L. paracasei*, *L. sakei* and *L. curvatus* strains from the LAB groups used in this study have never been investigated for the utilization of commercial resistant starch products. The prebiotic capacity of resistant starch types varies according to the resistant starch type and bacterial strain. Although the resistant starch type starch used in the studies was produced commercially or isolated from food, it was utilized by lactic acid bacteria. Current studies in the literature indicate that commercially resistant starch types could be utilized by various LAB and that resistant starches could be prebiotic. In this study, it was determined that lactic acid bacteria utilize commercial resistant starch types as prebiotics, consistent with the literature. Resistant starch is utilized by lactic acid

bacteria as a carbon source instead of glucose. As the utilization of resistant starch types by bacteria increased, the prebiotic capacity also increased.

CONCLUSION

Resistant starch is recognized as a prebiotic ingredient. However, for resistant starch types to be accepted as prebiotic components, it is necessary to determine by which bacteria they are utilized. At the end of the study, it was determined that Hi-maize 260, Novelose 330 and Demirpolat-resistant starches utilized by LAB were prebiotic. Considering the data obtained, it has been revealed that all the LAB strains and type cultures used in this study can be use with three different types of commercial-resistant starch as prebiotics. In addition, the prebiotic capacity of Demirpolat type 4 resistant starch produced in Turkey was examined for the first time. These commercial-resistant starch types have the prebiotic capacity that can be used in the food industry to contribute to the development of new functional foods. After determining the functional properties of lactic acid bacteria isolated from the foods included in the study, they can be included in the diets of individuals as potential probiotics. When commercially produced resistant starches are used with the bacteria involved in the work, a symbiotic component can be created. These results will lead to the emergence of new products that can potentially be used as prebiotics, probiotics, or symbiotic in the food industry.

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