Fermentation of Native Wheat, Potato, and Pea Starches, and their Preparations by *Bifidobacterium* – Changes in Resistant Starch Content

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Abstract

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The capability was studied of the selected *Bifidobacterium* strains to utilise the resistant starch fraction (RS) from native starches of the following origin: wheat, potato, and pea, and their preparations obtained experimentally by physical and enzymatical modifications. Furthermore, the potential influence of the gelatinisation process on the degree of utilisation of RS from the investigated starch samples was studied. The following strains: *B. pseudolongum* KSI9, *B. animalis* KS20a1, and *B. breve* KN14, were chosen. The native starches and their preparations were characterised by their different contents of the RS fraction, which was metabolised during *in vitro* fermentation for *Bifidobacterium* growth. The highest decrease in the RS content was observed in the case of native potato and pea starches after 24-h fermentation by *Bifidobacterium* strains. The RS fraction of the wheat starch preparation was generally a better substrate for the selected bacteria (19–34%) in comparison with the native wheat starch (0–13%). The gelatinisation process of the native starches and their preparations had a negligible effect on the RS fraction utilised as a substrate for stimulating the growth of the *Bifidobacterium* strains selected.

Keywords: starch modification; *in vitro* fermentation; starch gelanisation

Among all the bacteria colonising the intestine, particular attention should be paid to *Bifidobacterium,* which is one of the largest groups of saccharolytic bacteria constituting ca. 25% of the total population of bacteria present in the intestines of adults and ca. 95% of those in the intestines of infants (Gibson & Roberfroid 1995). Bifidobacterial numbers in the human gut tend to decrease with age, but they can be increased either by continuous ingestion of bifidobacteria-containing preparations or food supplementation with substrates (bifidogenic factors or prebiotics) that promote the growth of endogenous bifidobacteria in the gut (Alander *et al*. 2001). The major substrates available for microflora fermentation are: oligosaccharides (DP 3–9), non-starch polysaccharides of the plant cell wall (DP > 9 i.e. hemicelluloses, pectins) as dietary fibre components, and the starch fraction resistant to enzymatic hydrolysis in the upper part of the gastrointestinal tract (Tomomatsu 1994; Craig *et al*. 1998). It is known that the amounts of digested and absorbed starch are different, with the non-digested part of starch being transferred to the colon. It is estimated that approximately 8–10% of starch consumed daily is not digested and reaches the large bowel (Cummings & MacFarlane 1997). The amylase-resistant starch (RS) is acknowledged as an efficient substrate undergoing fermentation by the microflora colonising the large intestine.

Bacteria such as certain species of *Bifidobacterium* metabolise undigested polysaccharides such as resistant starch, using this component as a source of carbon and energy necessary for the growth (Arrigoni *et al*. 2002). The resistant starch fraction exhibits some potential physiological effects such as improved glycaemic and insulinaemic responses, improved bowel health, improved blood lipid profile, increased satiety, and reduced energy intake (Soral-Śmietana & Wronkowska 2004; Nugent 2005).

Our previous studies demonstrated that the applied procedure for starch modification including: autoclaving, recrystallisation (cooling), and enzymatic hydrolysis by thermostable α-amylase strongly influenced the physicochemical properties of these preparations in comparison with native starches (Wronkowska *et al*. 2004). The starch preparations used in the present study showed an atypical crystalline structure irrespective of the starch origin and revealed different thermal characteristics compared with native starches. The results obtained in differential scanning calorimetry showed that the peak temperature for starch preparations ranged between 128°C and 140°C, but the conclusion temperature ranged from 143°C to 156°C (Wronkowska *et al*. 2004).

In this study, we investigated the possibility of using the resistant starch fraction from native starches and their experimental preparations with different resistant starch contents as substrates for the selected *Bifidobacterium* strains. Furthermore, the potential influence of the gelatinisation process on the degree of utilisation of RS from the starch samples investigated was also studied.

MATERIAL AND METHODS

Material. Native industrially isolated commercial starches: wheat, potato, and pea starches defined as reference samples and constituted the material for experimental modifications. The starch preparations were obtained on a laboratory scale according to Wronkowska *et al*. (2006). The starches were suspended in distilled water (1:3.5), autoclaved (121°C/1 h), and cooled (4°C/12 h). Following 45 min hydrolysis with thermostable α-amylase (0.4 ml Termamyl/1 g of starch), the respective sample was autoclaved (120°C/20 min) to inactivate the enzyme. After autoclaving, the sample was washed several times with distilled water (sample:water, 1:5) to remove soluble α-glucans. The modified preparations obtained were freeze-dried and powdered to particles < 400 μm.

Methods. In our previous investigation, it was found that starches were fermented only by certain strains belonging to the species: *Bifidobacterium animalis*, *Bifidobacterium breve*, and *Bifidobacterium pseudolongum* (Soral-Śmietana *et al*. 2005). On the basis of those results, three *Bifidobacterium* strains were used for analyses: *B. pseudolongum* KSI9, *B. breve* KN14, and *B. animalis* KS20a1. The tested strains of *Bifidobacterium* were obtained from the collection of Microbiological Laboratory of the IAR&FR PAS (Olsztyn, Poland). The gelatinisation temperature of the native starches was determined in a Brabender apparatus. The hydrothermal process provoking the gelatinisation of the native starches was run 5 min at individual temperatures: 65°C for wheat, 60°C for potato, and 68°C for pea starches. The starch preparations were heated at 80°C for 10 min in a water bath with minimal stirring. The native starches and their preparations were sterilised in a thin layer with UV (125 μW/m^2) for 15 min to inactivate the microflora. The modified liquid Garche's medium (without sugar) (Rasic 1990) containing 1% of native starches or their preparations (non-gelatinised or gelatinised) was inoculated with ~10^5 of the selected strains of bifidobacteria and incubated at 37°C/24 h under anaerobic conditions. After 24-h of the microbiological fermentation, the samples were freeze-dried and the content of resistant starch was determined. All determinations were made in three replications.

The resistant starch (RS) analysis was carried out on the samples according to Champ *et al.* (1999) method. The resistant starch is the starch not hydrolysed by pancreatic α-amylase. The products of hydrolysis, solubilised in 80% ethanol, were discarded. The resistant starch present in the pellet was solubilised in 2 mol/l KOH, and then hydrolysed into glucose with amyloglucosidase. Glucose was then quantified with a glucose oxidase/peroxidase analysis kit (Liquick Cor-GLUCOSE 120; PZ, Cormay S. A., Lublin, Poland). The results of the resistant starch content determination are given as the mean and the standard deviation of three independent measurements.

The degree of RS utilisation was determined as a difference between the contents of the resistant starch fraction in the samples before and after fermentation by the individual *Bifidobacterium*

strains. The utilisation was expressed in percentages.

Scanning electron microscope (JSM 5200, Tokyo, Japan) micrographs were obtained after spraying freeze-dried samples with gold and visualised at an acceleration of 2; 5 or 10 KeV.

RESULTS AND DISCUSSION

The materials which were tested *in vitro* as substrates for *Bifidobacterium* strains demonstrated different contents of resistant starch (Table 1). The RS content in native starches of different botanical origins ranged from 3.1% to 60.8% in dry matter (DM). The modification used caused a considerable increase in the resistant starch content in the starch preparations obtained from 63.5% to 72.7% in DM. The resistant starch formation is a complex process that involves several processes taking place simultaneously. In different crops, Yadav *et al*. (2009) found a significant interaction between the heating/cooling treatment and increase in the resistant starch fraction content. The percentage increase in the RS content of repeatedly heated/cooled tubers was smaller in comparison with that of legumes and cereals. Soral-Śmietana *et al.* (2005) showed that the resistant starch content in physically-modified starch preparations obtained by cold syneresis of starch gels according to Lewandowicz *et al*. (1998) ranged from 7.7% in wheat preparation to 18.6% in potato preparation.

Microstructure of granules of native starches: wheat, potato, and pea starches (Figures 1a, 2a, and 3a) differed in shape and size. The modification of the starches, which included autoclaving, recrystallisation, and enzymatic hydrolysis by thermostable α-amylase, changed significantly the native structure of the starch granules and the microstructure of these starches preparations as shown in Figures 1b, 2b, and 3b. The micrographs of the starch preparations showed small particles of oval or spherical shapes, which formed larger clusters and revealed a tendency to associate. This transformation of the structure made them resistant to amylolytic attack. Yadav *et al.* (2009) showed that the granular structure of native starches disappeared upon the resistant starch formation by repeated heating/cooling treatment. That new structures demonstrated larger irregularly shaped particles with a sponge-like porous network. The microstructure of the physically-modified starch preparations obtained by cold syneresis of starch gels (Lewandowicz & Soral-Śmietana 2004; Soral-Śmietana *et al*. 2005) revealed a structure similar to the pregelatinised or extruded starch looking like an integrated gel, which was the composition of two-fractions, amylose and amylopectin.

The resistant starch content decreased after 24-h hydrolysis with the selected *Bifidabacterium* strains in all starches investigated with the exception of native wheat starch (Table 1). Generally, the resistant starch fraction of native starches was preferentially used by the investigated bacteria in

Table 1. Content of resistant starch fraction in native and modified starches before and after 24-h fermentation by selected *Bifidobacterium* strains

Sample	Resistant starch content before fermentation (% DM)	Resistant starch content after 24-h fermentation (% DM)					
		B. pseudolongum KSI9		*B. animalis* KS20a1		*B. breve* KN14	
		non-gelatinised	gelatinised	non-gelatinised	gelatinised	non-gelatinised	gelatinised
Wheat starch							
native	3.1 ± 0.2	3.0 ± 0.5	2.9 ± 0.3	3.1 ± 0.4	3.1 ± 0.3	3.0 ± 0.5	3.1 ± 0.2
preparation	63.5 ± 1.6	42.1 ± 0.4	41.4 ± 0.2	42.1 ± 0.6	41.4 ± 0.2	51.2 ± 0.5	50.9 ± 0.7
Potato starch							
native	60.8 ± 2.9	30.3 ± 1.0	24.6 ± 1.6	25.6 ± 1.0	22.6 ± 1.3	35.1 ± 0.6	19.2 ± 1.0
preparation	67.7 ± 1.0	47.7 ± 0.7	45.3 ± 1.2	48.7 ± 0.7	48.2 ± 1.0	49.1 ± 0.6	48.2 ± 1.0
Pea starch							
native	31.5 ± 1.6	10.5 ± 0.7	9.9 ± 0.9	9.8 ± 0.9	8.2 ± 1.0	8.8 ± 0.9	7.3 ± 1.5
preparation	72.7 ± 1.0	50.9 ± 0.7	49.3 ± 1.2	52.3 ± 0.6	52.0 ± 1.0	56.4 ± 0.6	54.4 ± 1.0

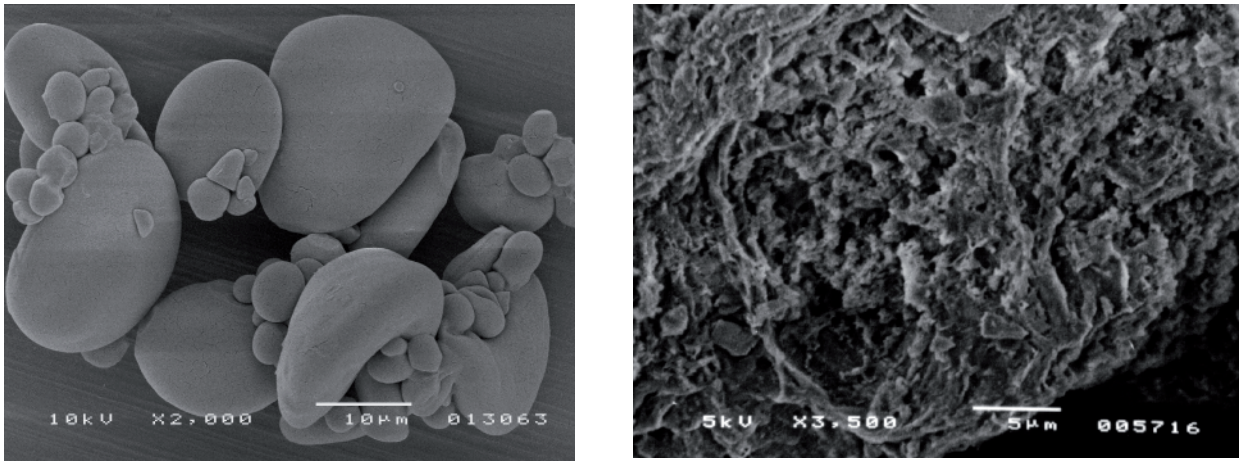

Figure 1. SEM-microstructure of (a) native wheat starch and (b) wheat starch preparation

the range of 42–72% (Table 2). The starch fraction resistant to amylolysis in native wheat starch was utilised by the investigated *Bifidobacterium* strains only in a negligible degree. However, the modification used for obtaining wheat starch preparation visibly influenced the utilisation of RS. The investigated *Bifidobacterium* strains: *B. pseudolongum* KSI9, *B. animalis* KS20a1, and *B. breve* KN14 utilised from 19.9 to 34.9% of RS from wheat starch preparation (Tables 1 and 2). The resistant starch fraction from potato and pea starch preparations was utilised to the same degree of about 30%. The results obtained for those two preparations provided distinctly lower values compared to those for native potato and pea starches. Wronkowska *et al*. (2006) found that the growth and acidifying activity of the selected strains of *Bifidobacterium* were higher in fermented modified starches, obtained using the same modification procedure as in this study, compared to native starches. The utilisation of the resistant starch from physically modified preparations, obtained from tapioca and normal and waxy corn, was generally higher compared to that of native starches (Wronkowska *et al*. 2008).

The gelatinisation of native starches had an insignificant effect on the utilisation of the RS fraction (Tables 1 and 2). It was found that the utilisation of the resistant starch from the starch preparations examined was at the same level as in the non-gelatinised preparations. With native starches, the utilisation of the RS fractions was higher compared to that of non-gelatinised native starches. Hydrothermal processes occurring during starch gelatinisation are one of the factors which influence starch digestion and absorption in gastrointestinal tract (Singh *et al.* 2010). This is the reason why this factor (gelatinisation) was used in this study to modify the structure of starches and their preparations. During gelatinisation, the starch granules

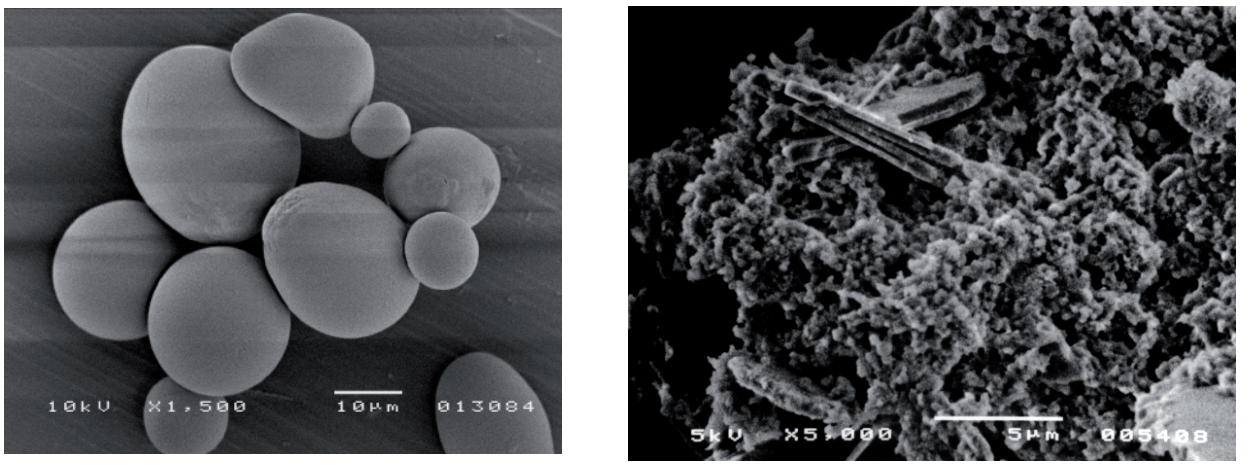

Figure 2. SEM-microstructure of (a) native potato starch and (b) potato starch preparation

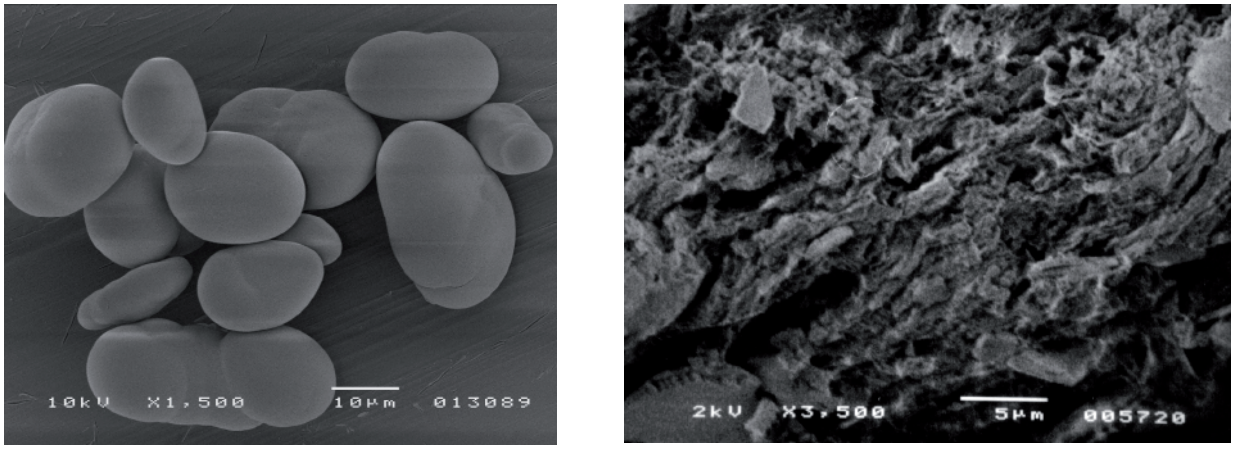

Figure 3. SEM-microstructure of (a) native pea starch and (b) pea starch preparation

Table 2. Degree of utilisation of the resistant starch fraction from native and modified starches by selected *Bifidobacterium* strains after 24-h fermentation

Sample	Degree of utilisation of the resistant starch (%)					
	B. pseudolongum KSI9		*B. animalis* KS20a1		*B. breve* KN14	
	non-gelatinised	gelatinised	non-gelatinised	gelatinised	non-gelatinised	gelatinised
Wheat starch						
native	3.2	6.5	0.0	0.0	3.2	0.0
preparation	33.2	34.8	33.7	34.8	19.4	19.8
Potato starch						
native	50.2	59.5	57.9	62.8	42.3	68.4
preparation	29.5	33.1	28.1	28.8	27.5	28.8
Pea starch						
native	66.7	68.6	68.9	74.0	72.1	76.8
preparation	30.0	32.2	28.1	28.5	22.4	25.2

absorb water, swell, and become more susceptible to enzymatic degradation (Rooney & Pflugfelder 1986). This phenomenon was observed for native starches but not for their preparations.

CONCLUSION

Bifidobacterium strains selected for this study: *B. pseudolongum* KSI9, *B. animalis* KS20a1, and *B. breve* KN14 used the native starches from three origins and their preparations as a source of carbon and energy for their growth. Generally, the resistant starch fractions from native starches were better substrates and their utilisation was higher compared to the modified starch preparations. A significant decrease in the level of resistant starch was observed in native potato and pea starches and their preparations after 24-h fermentation with the *Bifidobacterium* strains examined in the experiment. However, the gelatinisation process of native starches and their preparations had a negligible influence on the resistant starch metabolism by the selected bifidobacteria.

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