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PHYSICALLY-MODIFIED WHEAT OR POTATO STARCHES, THEIR PHYSICO-CHEMICAL PROPERTIES AND METABOLISM BY *BIFIDOBACTERIA*

Summary

The aim was to study the effect of physical processes (autoclaving/cooling cycles and spray drying) on starches having different crystalline structure: wheat (type A) or potato (type B) starch. First, the extent of changes in the physico-chemical properties of these physically-modified starches as the carbon and energy sources for growth in *in vitro* conditions was investigated.

Characteristics of functional properties, e.g. water binding capacity (WBC), indicated that both native starches had low affinity to water, that increased however 4-times after modification. The opposite tendency was observed for fat absorption (FA). Viscosity of water dispersion dramatically decreased after modification of both starches.

The ability of the tested *Bifidobacterium* strains to metabolise native or physically- modified wheat and potato starches was differentiated. *B. pseudolongum* KSI9 and *B. animalis* KSD29a3 isolated from animals utilised the examined starches as easily accessible substrates of fermentation, whereas *B. breve* ATCC 15700 isolated from human did not metabolise or only negligibly fermented starch preparations. The number of bifidobacteria populations as well as their acidifying activity were higher in the media containing wheat starch in comparison to the potato starch, whereas no significant differentiation was observed between the results obtained in media with native or modified starch.

The results suggest that native or experimentally-modified wheat and potato starches with some fraction of resistant starch can be a good substrate for colonic bifidobacteria.

Introduction

Botanical origin of native starch determines different diffraction patterns, i.e. A-type and B-type. When crystallising linear chains in solution, during the *in vitro* studies, the A-type is favoured kinetically and B-type thermodynamically [11]. The struc-

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ture of the A-type is obtained preferentially under conditions of high crystalisation temperature, high polymer concentration, and short chain length [12]. For native starches, amylopectin molecules from A-type starches have shorter constitutive chains and higher numbers of short-chain fractions than those from B-type starches [15]. It seems that mechanism of the retrogradation process is analogous to that found for many other helix-forming polysaccharides. This stems from the central role of double helix formation in either amylose or amylopectin retrogradation behaviour. For long amylose chains, gelation and related network properties are a direct result of multiple helix formation creating a meshwork of cross-links between chains in an exactly analogous mechanism to e.g. gelatin or agar [13]. For amylopectin, the analogies are fewer due to the unusual clustering of relatively short branches. Complexity increases when mixtures of amylose and amylopectin are being retrograded. As a result of physical modification, the following changes can be observed in the granules of native starches: swelling, gelatinisation, solubilisation, retrogradation. This distributional heterogenity has an impact on functional properties.

The large bowel harbours nutritionally and physiologically diverse range of bacteria, promoting normal intestinal function, and offering the host protection against infections [18]. Bifidobacteria are generally considered beneficial for human health and together with lactobacilli are widely used in probiotic preparations and foods. Several positive effects have been related to bifidobacteria. These include synthesis of vitamins, supplementation in digestion and absorption, inhibition of growth of exogenous organisms, and stimulation of the immune system. Bifidobacterial numbers in the human gut tend to decrease with age [16]. To maintain a high level of bifidobacteria in the gut a two-fold strategy can be applied. Numbers of bifidobacteria can be increased either by continuous ingestion of bifidobacteria-containing preparations or foods, or food can be supplemented with substrates (bifidogenic factors or prebiotics) that specifically promote the growth of endogenous bifidobacteria in the gut [14].

Amylose-resistant starch is considered a very good substrate easily fermented by microflora of the large bowel. This fermentation is of significant importance when it comes to environment and functioning of this part of the alimentary tract. Apart from gas products (hydrogen and methane), short-chain fatty acids (acetic, propionic, and butyric) are produced [7]. Proportion between short chain fatty acids, e.g. acetic, propionic and butyric, produced during 24-hour fermentation *in vitro* has been indicated to depend on the origin of resistant starch [21]. As a substrate for rats intestine microflora, resistant starch from wheat and potato can affect the pH lowering of fermented medium and that of potato origin can be a source of valeric acid during fermentation over 12 hours. It was stated that during fermentation of amylose-resistant starch relatively higher amount of short-chain fatty acids is formed, compared to fermentation of dietary fibre fractions [6, 9, 22]. It has been also suggested that at simul-

taneous fermentation of resistant starch and dietary fibre, the starch undergoes fermentation first [8].

The aims of the study were to determine how the gelatinisation, retrogradation and dehydration in stream of hot air affect the properties of wheat or potato starches, and whether native or modified starches can be a substrate for the growth of certain *Bifidobacterium* strains that inhabit the human or animal intestine.

Material and methods

Material

Commercial wheat and potato starches were investigated. The modification was made in a laboratory scale using such physical processes as autoclaving/cooling cycles and spray-drying for dehydration, according to technological procedure described earlier [24].

Methods

Chemical components of native and modified starches were determined as follows: nitrogen by the Kjeldahl method and ash after mineralisation in muflon oven at 700°C according to the AOAC standard chemical methods [1]. The content of elements was analysed according to AAS method, after wet mineralisation by the mixture of nitric and perchloric acids.

The amylose content was determined according to Morrison et al. [17]. The resistant starch analysis was carried out using the Champ method [4]. Functional properties as water binding capacity (WBC) and fat absorption (FA) were also analysed [19]. The course of gelatinisation was followed with Brabender viscograph procedure under the following conditions: 8% water dispersion was measured in cartridge 700 cmg; heating/cooling (25–95°C) with the rate of 1.5°C/min; thermostating for 30 min.

The following strains of *Bifidobacterium* were tested: reference strain *B. breve* ATCC 15700, and *B. pseudolongum* KSI9 as well as *B. animalis* KS29a3 all isolated in the Department of Food Microbiology. They were selected in the preliminary studies on the basis of the ability to utilise starch using standard API 50CH test (BioMerieux) and commercial potato starch. Starch utilisation was evaluated by the following criteria: growth and acidifying activity of bifidobacteria for the examined substrates, compared with glucose. The ability of *Bifidobacterium* strains to metabolise native or modified, wheat or potato starches was studied in liquid minimal media, which contained meat peptone 1% (w/v), L-cysteine hydrochloride 0.04% (w/v), buffering salts and essential ions, final pH 6.4 [2]. After dispersing 1% (w/v) of glucose or the examined starch preparations in the medium, they were immediately heat-treated (95°C/10 min). The media were inoculated with active bifidobacteria cultures

(~$1 \cdot 10^7$ cfu/mL) and incubated at 7°C for 24 h in anaerobic conditions (pyrogallol stoppers). After incubation, the number of bifidobacteria was determined in modified Garche's medium [2] using the plate technique. The plates were incubated at 37°C for 48 h in anaerobic jars – Oxoid Anaerobic System with Gas Pak H_2+CO_2. Acidifying activity, as pH of fermentation medium, was determined for each strain.

Results and discussion

Analysis of the chemical composition of the investigated material indicated that, as a result of the modification process used, only negligible part of amylose fraction was released (tab. 1). However, the content of resistant starch decreased, compared to natural starches prior to modification. As a result of gelatinisation and retrogradation, the III-type resistant starch was obtained, while in native starch – type II RS is present [20].

Table 1

Chemical composition of native and modified starches.

Sample	Amylose [% d.m.]	Nitrogen [% d.m.]	Ash [% d.m.]	Resistant starch [% d.m.]
Wheat starch:				
native	21,34	0,04	0,25	22,22
modified	19,08	0,12	0,26	7,76
Potato starch:				
native	28,75	0,03	0,45	61,60
modified	26,72	0,15	0,41	8,58

Table 2

Content of microelements in native and modified starches.

Sample	Content of elements [μg/g d.w.]								
	Ca	Mg	Na	K	P	Cu	Mn	Fe	Zn
Wheat starch:									
native	83.45	17.30	220.40	103.37	473.51	0.10	0.29	0.90	0.99
modified	267.13	42.05	271.31	130.04	513.93	4.83	0.49	11.03	5.23
Potato starch:									
native	416.02	63.61	17.21	54.91	581.68	0.15	0.53	1.55	0.42
modified	678.59	98.56	51.99	90.17	663.78	1.16	0.89	8.31	12.13

Therefore, physiological process is connected with a different substrate what is clearly classified from the nutritional point of view [10]. Physically-modified starches

were characterised by a higher content of macroelements: Ca, Mg, and Na, compared to native starches (tab. 2). The content of other elements also increased in starches subjected to modification, what was connected with changes in the granular structure of starch as well as with production of heterogeneous mixture of amylose and amylopectin as a consequence of the technological processes applied.

Modification of the investigated starches caused a change in their character, compared to native starches which gained hydrophilic character (fig. 1). On the basis of Brabender viscosity curves (fig. 2, 3), it was stated that the course for native starches was typical of that kind of starches. In the case of modified wheat and potato starches the curves were typical for pregelatinised starches. As a result of the physically process used, low viscosity obtained for dispersion of modified starches indicated the significant changes of that properties of investigated starches.

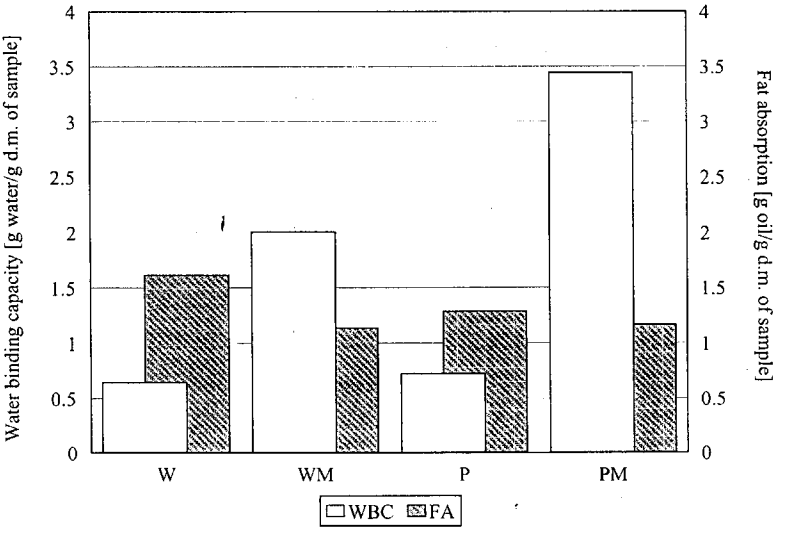

Fig. 1. Functional properties of native and modified starches:
W – native wheat starch, WM – modified wheat starch, P – native potato starch, PM – modified potato starch.

Strains of *Bifidobacterium* were tested *in vitro* for the metabolism of native and modified starches to the growth of population (tab. 3). In the control medium containing glucose as a good source of carbon and energy for bifidobacteria, the growth of the tested strains was differentiated. After 24 h of incubation the number of *B. breve* $1.8 \cdot 10^9$ cfu/ml was the highest and accompanied by the highest reduction of pH to the level of 4.6. The population numbers of the other strains – *B. animalis* and *B. pseudolongum* – were lower, $3.8 \cdot 10^8$ and $1.7 \cdot 10^8$cfu/ml, respectively, and the pH values of these cultures were reduced only to the level of 5.3 and 5.0, respectively.

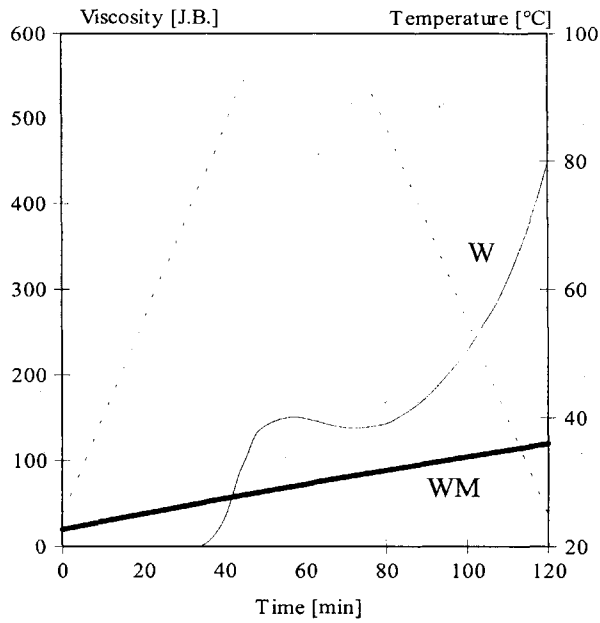

Fig. 2. Brabender viscosity curves of native (W) and modified (WM) wheat starches.

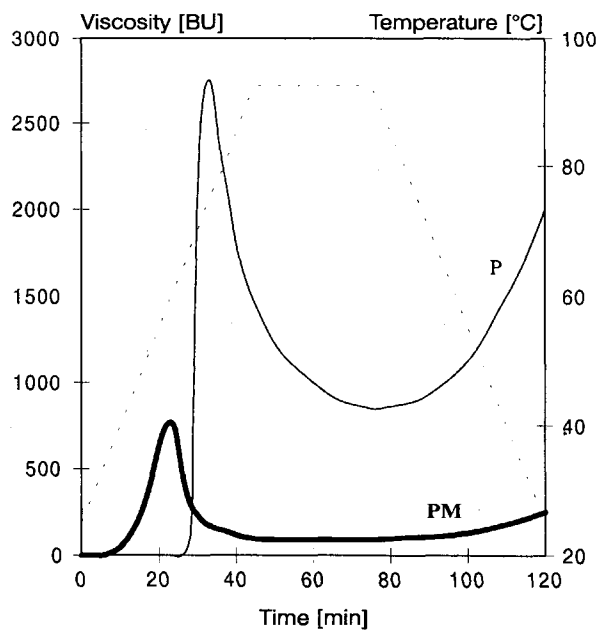

Fig. 3. Brabender viscosity curves of native (P) and modified (PM) potato starches.

Table 3

Growth and acidifying activity of bifidobacteria.

Substrate	*B. breve* ATCC 15700		*B. pseudolongum* KSI9		*B. animalis* KS29a3	
	Number [cfu/ml]	pH	Number [cfu/ml]	pH	Number [cfu/ml]	pH
Glucose	$1.8x10^9$	4.6	$1.7x10^8$	5.0	$3.8x10^8$	5.3
Wheat starch:						
native	$7.5x10^7$	6.0	$2.0x10^8$	5.0	$4.6x10^8$	5.1
modified	$2.2x10^7$	6.0	$1.9x10^8$	4.9	$4.0x10^8$	5.5
Potato starch:						
native	$5.5x10^6$	5.9	$1.4x10^8$	5.0	$3.2x10^8$	5.5
modified	$2.1x10^7$	6.0	$1.1x10^8$	5.1	$2.8x10^8$	5.6

Inoculum of bifidobacteria strains ~$1x10^7$ cfu/ml; after inoculation pH ~6.2; 24-h anaerobic culture with 1% of examined starch substrates or glucose as control.
The results are means of 3 determinations.

In opposite to the control, in the cultures with the examined starches as a substrate instead of glucose, the number of *B. breve* population remained close to the inoculum level. The pH value slightly lowered to the level of ~6.0. The results indicated only negligible or lack of fermentation of the starches by *B. breve* strain isolated from human. The number of *B. pseudolongum* populations in the cultures with starch preparations ranged from $1.1 \cdot 10^8$ to $2.0 \cdot 10^8$ cfu/ml and the pH level was close to 5.0, which was comparable to the results in the control medium with glucose. In the cultures of *B. animalis* with starches as a substrate, the population numbers ranged from $2.8 \cdot 10^8$ to $4.6 \cdot 10^8$ cfu/ml, and were comparable to the control, whereas pH value, ranging from 5.1 to 5.6, was generally lower than in the control. The results indicate that the strains of *B. animalis* and *B. pseudolongum* isolated from animals utilised the examined starches as easily accessible substrates of fermentation.

The ability of starch utilisation seems not to be a common feature within genus *Bifidobacterium*. As a result of selection of 40 *Bifidobacterium* strains to complement resistant starch in a synbiotic yoghurt, Crittenden et al. [5] revealed that only 1 strain *B. lactis* Lafti™ B94 (closely related to *B. animalis*) could hydrolyse resistant starch. Our preliminary results indicated that the starches were metabolised only by several strains isolated from animals and, in lower extent, from infants [data not published]. They belonged entirely to the species *B. animalis*, *B. pseudolongum* and *B. breve*. While examining *in vitro* the utilisation of amylopectin and high-amylose maize starch granules by human colonic bacteria, Wang et al. [23] showed that only 6 out of 36 cultures tested utilised maize-starch amylopectin. The authors demonstrated that *Bifidobacterium* spp., *Bacteroides* spp., *Fusobacterium* spp., and strains of *Eubacterium*,

Clostridium, *Streptococcus* and *Propionibacterium* could hydrolyse the gelatinised maize-starch amylopectin, while only *Bifidobacterium* spp. and *Clostridium butyricum* could efficiently utilise high-amylose maize starch granules.

The use of starch as substrate enhancing bacterial fermentation in the colon is promising but also very difficult area of studies, because the examined material is not uniform, since it contains digestible as well as non-digestible compounds in the upper gastrointestinal tract. Our previous studies *in vivo* on the effect of different non-digestible preparations on the gut microecosystem of rats showed that modified corn starch significantly increased the bifidobacteria number by 1.2 log cfu/g of faeces [3]. Unfortunately, the preparation also increased the values for markers of unhealthy caecal changes ($N\text{-}NH_3$ content and β-glucuronidase activity). The development of starch preparations selectively stimulating the growth of bifidobacteria needs further research.

Conclusions

As a result of physical modification, native wheat and potato starches lost their granular structure and both modified starches were different from the native ones in the affinity to water, viscosity and gelatinisation.

The strains of *B. animalis* and *B. pseudolongum* isolated from animals utilised starch as a fermentation substrate, whereas the strain *B. breve* of human origin fermented only negligibly or not at all the examined preparations of wheat and potato starches. The bifidobacteria population number as well as medium acidification were higher in the media containing wheat starch than the potato starch.

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SKROBIE FIZYCZNIE MODYFIKOWANE PSZENNA I ZIEMNIACZANA, ICH WŁAŚCIWOŚCI FIZYKOCHEMICZNE ORAZ METABOLIZOWANIE PRZEZ BIFIDOBAKTERIE

Streszczenie

Celem badań było określenie wpływu procesów fizycznych (cykle autoklawowania/chłodzenia, suszenie rozpyłowe) na skrobie posiadające odmienną strukturę krystaliczną: pszenna (typ A), ziemniaczana (typ B). Badany był zakres zmian właściwości fizykochemicznych skrobi poddanych modyfikacji fizycznej, które stanowiły źródło węgla i energii do wzrostu bifidobakterii w warunkach *in vitro*.

Charakterystyka właściwości funkcjonanych, takich jak zdolność wiązania wody (WBC), wskazała, że obie naturalne skrobie wykazywały niskie powinowactwo do wody, które po modyfikacji zwiększyło się ok. 4-krotnie. Zauważono przeciwną tendencję w przypadku absorpcji oleju (FA). W wyniku ogrzewania zawiesiny wodnej badanych skrobi w aparacie Brabendera stwierdzono znaczące obniżenie lepkości wskutek modyfikacji obu skrobi.

Zdolność wybranych szczepów *Bifidobacterium* do metabolizowania naturalnych bądź fizycznie modyfikowanych skrobi pszennej i ziemniaczanej była zróżnicowana. *Bifidobacterium pseudolongum* KSI9 i *Bifidobacterium animalis* KS29a3, izolowane od zwierząt, wykorzystywały badane skrobie jako łatwo dostępny substrat do fermentacji, natomiast *Bifidobacterium breve* ATCC 15700, izolowany od człowieka, nie metabolizował, lecz nieznacznie fermentował badane skrobie. Liczebność populacji bifidobakterii, jak również ich aktywność kwasząca, były wyższe na podłożu zawierającym skrobię pszenną w porównaniu ze skrobią ziemniaczaną. Jedynie nieznaczne zróżnicowanie obserwowano w tych wskaźnikach *in vitro* pomiędzy skrobiami naturalnymi i modyfikowanymi.

Uzyskane wyniki sugerują, że naturalne i modyfikowane skrobie, pszenna i ziemniaczana, zawierające frakcję amylazooporną mogą stanowić dobry substrat dla bifidobakterii zasiedlających jelito grube.