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RESISTANT STARCH – NUTRITIONAL AND BIOLOGICAL ACTIVITY

Maria Soral-Śmietana, Małgorzata Wronkowska

Division of Food Science, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland

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This paper presents different nutritional and biological activities of resistant starch in human organism. Resistant starch fractions may indicate some physiological functions remaining in relation with their physical and physico-chemical properties, including water holding capacity, intestinal transit time, potential glycaemic index, insulin action, induction of thermogenesis (gases, ammonia, phenols), mineral absorption, intestinal pH. Colonic function, especially bowel habit, short-chain fatty acids production, N-metabolism, bacterial activity and epithelial cell function, are largely controlled by carbohydrates that enter the colon as well as, the resistant starch (RS), non-starch polysaccharides (NSP) and oligosaccharides. The relationship between diet, microflora and colorectal cancer is complex and intimate. Substances entering the colon from ileum and the resident microflora are major determinants of colonic physiology. These, together with the innate biology of the colon, are pertinent to the initiation and promotion of colorectal cancer. The biological activity of resistant starch, as it results from the recent literature data, can also positively influence the prevention of gastrointestinal disease.

INTRODUCTION

The most abundant plant dietary carbohydrates are starches of different origin. In food they occur usually as a structure stabilizer. In solutions, the starch fractions, amylose and amylopectin, can easily undergo hydrolysis forming a mixture of simple oligosaccharides and limit branched dextrins. After the time when carbohydrates were considered only as the energetic component of a diet, nowadays they are treated as a potential health-beneficial component for a man [Stephen, 1994; Tomomatsu, 1994; Asp *et al.*, 1996; Haralampu, 2000].

Depending on the degree of starch mechanical, physical or heat treatment during the technological processes used in the food manufacture, the range and the rate of starch hydrolysis are different and the role of starch in human digestive tract changes. The starchy food products may contain some starch categories, behaving differently in the human digestive tract according to classification proposed by Englyst *et al.* [1992, 1996]. These include: (1) readily digestible starch (RDS), *i.e.* digestion within 20 min; (2) slowly digestible starch (SDS), *i.e.* digestion within near 3 h; and (3) starch resistant to enzyme-catalysed digestion (RS), *i.e.* over 3–6 h. Recently a new category of enzyme resistant starch has been formulated, namely very resistant starch (VRS), which is not digested for a long period of time, up to 24 h and more. The range and the region of enzymatic hydrolysis of starch granules are affected by: (1) an increase in the ratio of external surface and solid phase volume; (2) modification of crystallinity as a result of gelatinisation and gel formation; (3) formation of complexes between amylose and amylopectin or depolymerisation of amylose and amylopectin. Such modification of starch is dependent on the conditions of a technological process and contribution of other components. The α-amylolysis of heterogeneous phases, as well as starch, requires the interpretation at the following stages [Colonna *et al.*, 1992]: (1) diffusion of enzyme molecule towards its substrate, (2) the porosity of starchy substrates, (3) the adsorption of enzymes on the substrate, and (4) catalytic event. After 24-h *in vitro* hydrolysis, depending on the origin of starches, significant differences in the manner and range of pancreatic α-amylase activity were noticed (Figure 1a–f) [Soral-Śmietana, 2000; Soral-Śmietana *et al.*, 2001b].

In the last decade, starch resistant to the hydrolytic activity of human digestive tract enzymes has attracted a lot of interest of nutritionists, physiologists and also physicians. At present, a variety of different starchy food products is available. Within the EURESTA programme, the resistant starch has been defined as "the sum of starch and products of starch degradation, that are not absorbed in the small intestine of healthy individuals" [Asp, 1992], and continuing is a major substrate for colonic fermentation and is a good source of butyrate.

Eearlier Englyst & Cummings [1985, 1986, 1987] identified "dietary starch" as potentially digested by α-amylase and "resistant starch", as being mostly derived from retrograded amylase, dextrins soluble in 80% ethanol and glucose. Schweizer *et al.* [1990] distinguished two categories of starch *i.e.* dietary starch, including low molecular weight dextrins and resistant starch, as it was defined by Englyst *et al.* [1992]. However the low molecular weight carbohydrates have been often considered as the products of the fermentation or hydrolysis by pancreatin amylase, that con-

Author's address for correspondence: Maria Soral-Śmietana, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, ul. Tuwima 10, 10-747 Olsztyn, Poland: tel.: (48 89) 523 46 51; fax: (48 89) 524 01 24; e-mail: soral@pan.olsztyn.pl

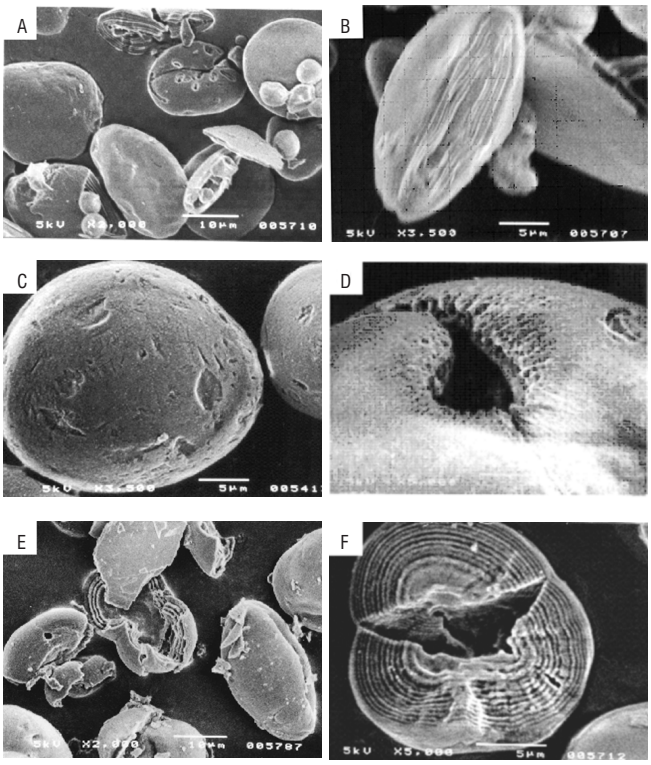

FIGURE 1. The native starches after 24-h *in vitro* hydrolysis with pancreatic alpha-amylase (SEM electronograms): A, B – wheat starch; C, D – potato starch; E, F – pea starch [Soral-Śmietana, 2000].

tinued the collection bag of ileal effluents inside [Englyst & Cummings 1985, 1986, 1987]. The continued hydrolysis after the collection of the ileostomy evaluates cannot be excluded, but it should be noted that low molecular weight material was identified also in ileal contents collected by intubation [Faisant *et al.*, 1993a]. Whereas up to now that part of soluble resistant starch cannot be measured by existing *in vitro* methods [Champ *et al.*, 2001].

Analysing starch during *in vivo* digestion and absorption in the small intestine of individuals Faisant *et al.* [1993a] has found the critical level of resistant starch composed of three structural types, *i.e.* (1) small amounts of oligosaccharides similar to the products of partial starch hydrolysis, (2) relatively high proportion of retrograded amylose, and (3) high molecular weight material corresponding to semi-crystalline starch fragments, probably present as the consequence of physical inaccessibility of starch and/or insufficient retention time in the intestine. Therefore, the retrograded and physically-inaccessible (or very slowly digestible) starch fractions seem to include many *in vivo* resistant starches in a normal diet. Botham *et al.* [1995] reported a wide range of molecular weight of the *in vivo* RS after meal containing retrograded starch gel, with the main fraction containing amylose fragments with DP 70–80. According to the *in vivo* research, after ingestion of various sources of RS [Faisant *et al.*, 1993 a, b; 1995 a, b], Champ [1995] ascertained the presence of three different RS fractions at the terminal part of the ileum: (1) the residues of starch granules and/or long chains of soluble starch molecules (DP>100); (2) the crystalline fraction with linear chains (DP about 26–38); and (3) oligosaccharides with DP 5 or less together with free glucose.

Asp *et al.* [1996] confirmed that the three main fractions of resistant starch are composed of: glucose and oligosaccharides, crystalline fraction with intermediate molecular size and of the high molecular weight fraction, containing residues of resistant granules as well as physically-enclosed starch. Somewhat varied estimations have been obtained regarding the molecular size of the intermediate fraction. The authors [Asp *et al.*, 1996] wondered if it represents a true variation in DP of starch fraction from various sources or whether it results from the methods used.

Resistant starch is assessed as a part of starch taken with mixed diet and considered as inert in transit through the small intestine section, but could be partly or completely metabolised through the fermentation in the large intestine [Bednar *et al.*, 2001]. The range and rate of fermentation and the possibility of energetic supply of microflora existing in that part of the digestive tract can vary. In many countries, including Poland, the daily diet is too rich in fat, most often unbalanced with carbohydrates and dietary fibre. Despite that resistant starch and non-starch polysaccharides (NSP) are connected with inert transit through the small intestine and fermentation in the large intestine, however the resistant starch should be regarded separately because of its chemical structure that differs radically from NSP.

The present nutritional challenges oblige to develop products with determined nutritional function, which would include both the dietary starches, with full or partial absorption degree, and some amount of starch resistant to the hydrolysis action of amylolytic enzymes.

CONTRIBUTION TO METABOLISABLE ENERGY

The resistant starch from heat-treated wheat starch, included to diets for rats, treated with antibiotics, was recovered in the feaces and when the RS in the diet increased, the utilisation of energy decreased significantly [Björck *et al.*, 1986]. Also the protein digestibility was not affected neither by the RS amount in the diets nor by the antibiotics. The authors concluded that the RS formed during the heat treatment was similar to easily fermentable dietary fibre.

Some studies show a net energy value of zero or less after consumption of poorly digested carbohydrate up to 70 g/d, on the average 8.4 kJ (2 kcal) digestible energy per 1 gram of RS fermented as the products of absorption during colonic fermentation [Behall & Howe, 1995; Ranhorta *et al.*, 1996]. Based on the metabolisable energy (ME) and breath hydrogen measurements, amylose and resistant starch from amylose appear to be utilised as energy sources by subject and by colonic bacteria [Behall & Howe, 1995; Wronkowska *et al.*, 2002a]. Fermentation appears to contribute significant to digestible energy when >20 g poorly digested carbohydrate per day is consumed and RS that reach the colon may also contribute to total energy.

PHYSICAL OR PHYSIOLOGICAL PROPERTIES

Resistant starch fractions may indicate some physiological functions remaining in relation with their physical and physico-chemical properties, including water holding capacity, intestinal transit time, potential glycaemic index, insulin action, induce thermogenesis (gases, ammonia, phenols), mineral absorption, intestinal pH.

Heijnen & Beynen [1997] presented the effect of resistant starch on the route of nitrogen excretion of cannulated pigs. Three groups of the animals were fed diets containing

uncooked resistant starch (RS_2), retrograded resistant starch (RS_3) or glucose. The pigs fed RS_3 had a significantly higher production of ileal digesta and faeces than the pigs fed glucose or RS_2. Dietary RS_2 and RS_3, in contrast to glucose, increased faecal nitrogen excretion, resulting of the reduced apparent nitrogen absorption.

Wronkowska *et al.* [2002b] showed the noticeable physiological effects comparing rats fed a diet containing 10% of native starches (wheat, potato, pea) of RS_2 type or their physically-modified preparations, containing RS_3 and the rats fed a diet with cellulose. After four weeks of the *in vivo* experiment on rats, an increase in the coefficient of protein efficiency ratio (PER) was observed. A significant decrease in nitrogen excretion by faeces or urine was noticed in rats receiving the native and modified starches with their diet. Additionally, the investigated starches caused statistically insignificant thickening of the caecum wall and diminishing caecal digesta, except for native potato starch. The results of Lopez *et al.* [2001] indicated that the caecal weight increased progressively, throughout the study on rats fed the diet with 20% of RS type 2, as well as on these fed raw potato starch and high amylose corn starch. The caecal pH of rats fed on the fibre-free diet was close to 7.0, however after 7 days of RS_2 incorporation to the caecum, the conditions were mainly acidic (pH 5–5.5). In our study [Wronkowska *et al.*, 2002b], being the result of 4-week caecum fermentation, the greater acidification of caecal digesta (range pH 6.7–5.8) was observed in comparison to cellulose (pH 6.8).

According to the Silvester *et al.* [1995], the resistant starch induced a decrease in ammonia levels in colon and serum as supported in humans.

Resistant starch is associated with the malabsorption of starch. Those results evidencing the decrease in postprandial glucose and insulin responses, have significant implications for RS utilisation in food formulations for people with certain forms of diabetes. Despite the beneficial effect of corn or rice resistant starch in drug-induced diabetic rats, under human type 2 diabetic conditions, it is feasible that such diabetes complications as hyperlipidemia can be controlled especially by rice resistant starch, however it might increase plasma glucose without changing insulin activity [Kim *et al.*, 2003].

According to Heijnen *et al.* [1995] studies on healthy men, the partial replacement of digestible starch by resistant starch (RS_2 – raw potato starch) effected the diet-induced thermogenesis (DIT), postprandial glucose and insulin responses. The raw potato starch begins to be fermented 6–7 h after consumption, which results in the rise of H_2 but not of CH_4 in the breathing out air, and the replacement of 27 g of digestible starch with RS_2 of the single meal – in lowering thermogenesis (DIT) by 90 kJ per 5 h on average. After the intake of raw starch, plasma glucose and serum insulin responses can be lower than these expected after the consumption of rapidly digested carbohydrate, because then both potent stimulators of insulin secretion, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), increase as suggested by Raben *et al.* [1994]. The decrease in insulin response after the consumption of the potato RS_2 in meal was explained by the substantial reduction of the diet-induced thermogenesis (DIT) [Heijnen *et al.*, 1995]. The interference with starch absorption implies also the long-term benefits in controlling hyperlipidemia.

The raw potato starch, as highly resistant to *in vitro* digestion (75% d.m.), was evaluated *in vivo* into lipid and glucose studied on healthy adults volunteers [Marchini *et al.*, 1998]. The results of fasting and postprandial blood were analysed taking into account the free fatty acids, glucose, insulin, triglyceride, cholesterol, chylomicron-triglyceride, within seven hours. A sporadic intakes of raw potato resistant starch did not improve postprandial blood glucose, and the main blood indicators did not differ statistically. However the improvement of the postprandial triglyceride metabolism by enhancing the clearance of chylomicron remnants was observed, that is usually considered as atherogenic particles. This finding is of interest to the coronary artery disease prevention. Marchini *et al.* [1998] suggested that the unexpected results and conclusions should be considered with caution, as the potato RS used in this study was probably more digested than in the *in vitro* study.

The effect of RS_2 diet supplementation with 30 g of raw green banana flour on the apparent absorption of minerals in the human small intestine was studied during ileostomy [Langkilde *et al.*, 2002]. This supplementation did not significantly change the ileal absorption of magnesium, calcium, zinc, sodium, or potassium, however it caused a small but highly significant reduction of iron in the ileal excretion. The results indicate that banana RS_2 did not influence the absorption of nutrients in the human small intestine, except for iron excretion which was slightly increased. The Langkilde *et al.* [2002] affirm that the ileostomy model seems to give the reliable results of *in vivo* RS measurement feasible. The studies of Heijnen *et al.* [1996] into magnesium absorption (apparent and true) which compared rats fed RS_2 *vs*. those fed RS_3 showed that RS_2 significantly enhanced apparent but not true magnesium absorption, as it lowered the faecal excretion of endogenous magnesium. The comparison of the effect of raw potato starch and high amylose corn starch, both RS type 2, included to rats' diet with to fibre-free diet on mineral utilisation (Ca, Mg, Zn, Fe, Cu) was investigated [Lopez *et al.*, 2001]. As a result of the hypertrophy of the caecal wall and the caecal acidification, the intestinal absorption of these elements, especially Ca, Mg, and Cu, increased.

The effect of resistant starch preparation from corn and rice on physiological indicators in diabetic rats was also studied [Kim *et al.*, 2003]. After three weeks of the *in vivo* experiment, a decrease in the intestinal transit time or the body weight were shown. The epididymal fat pads weight or weight of organs, *e.g.* thymus and spleen, decreased, however these of liver and kidney did not change. Kim *et al.* [2003] discussed that under the diabetic conditions gut physiology might be altered, and therefore it may be difficult to compare it to the normal state.

SHORT CHAIN FATTY ACIDS (SCFA)

In a world of rapidly changing food habits and stressful life style it is more and more recognized that a healthy digestive system is essential for overall quality of life. This is not surprising since the recognition that the intestinal tract is the organ with the largest surface and metabolic capacity of our body. It is the organ which absorbs the nutrients that are required for growth, development and health and excretes undesired and waste substances [Brouns *et al.*,

2002]. The large bowel plays a significant role in our immune defence against disease. The colon is a host to a large and diverse commensal flora of anaerobic bacteria. Colonic function, especially bowel habit, short-chain fatty acids production, N-metabolism, bacterial activity and epithelial cell function are largely controlled by carbohydrates that enter the colon. Numerous factors control fermentation and SCFA production in the large bowel. Substrate supply environmental conditions, *e.g.* pH and transit time [El Oufir *et al.*, 1996], relative proportions of major bacterial species and the metabolic activity of microflora [Macfarlane & Cummings, 1991] may all influence SCFA concentration and pattern.

Some carbohydrates of the diet avoiding the enzymatic digestion in the small intestine become the substrates of fermentation of the colon bacterial flora, as well as, the resistant starch (RS), non-starch polysaccharides (NSP) and oligosaccharides. They are fermented to gases, water and the main product of fermentation are short-chain fatty acids (SCFA), including mainly acetic, propionic and butyric acids. The above-mentioned SCFAs together with isobutyric and isovaleric acids originate also from bacterial degradation of undigested protein [Ferguson & Jones, 2000]. It was ascertained that during the fermentation of resistant to amylase starch fraction proportionally more SCFA are produced than during the fermentation of dietary fibre components [Englyst & Macfarlane, 1986; Cummings & Englyst, 1987; Stephen, 1994]. It has been suggested that the simultaneous occurrence of both components makes bacteria to the preferential fermentation of the starch resistant to amylase [Cummings *et al.*, 1996].

The produced SCFA are rapidly absorbed from the gut lumen, especially when the luminal pH is low or when there is a high concentrations of SCFA. About 95–99% of total produced SCFA is absorbed before reaching the rectum in a number of non-ruminant species [Von Engelhardt *et al.*, 1989]. SCFA are the weak acids with pKa of 4.79; 4.87 and 4.81 for acetate, propionate and butyrate, respectively. In normal colonic contents more than 95% of SCFA exist in an ionized form. Their major transport may occur in the undissociated, lipid-soluble form, and requires an equimolar disappearance of H^+ ions. Protonization requires three sources of H^+ ions: from digesta, from the dissociation of bicarbonate, and from Na^+- H^+ exchange or active K^+ - H^+ transport [Von Engelhardt *et al.*, 1998]. Studies on the isolated tissues, of animals and humans, have shown that different SCFA have the biological effects, either local or systemic, which have been suggested to be of benefit to health [Ferguson & Jones, 2000]. SCFA are assimilated by the mammalian host, and provide a high proportion of the total energy gained from the diet in herbivores, especially ruminants. In humans, the overall contribution of SCFA towards the host's energy requirement is far lower, but they have an important influence on colonic health. The main products of microbial fermentation in the large intestine can significantly influence their relative concentrations and production rates depending on diet and site of production, with typical ratios in faeces being around 3:1:1 acetate-propionate-butyrate [Cummings *et al.*, 1987]. The SCFA influence the decrease of pH of intestine. Therefore there exists the possibility of inhibiting cholate conversion and decreasing the amount of soluble deoxycholate with carcinogenic properties. In the large intestine, SCFA stimulate the resorption of water and sodium, thus limiting the risk of diarrhoea. In an acidic environment, they are capable of inhibiting the growth of some intestinal bacterial pathogens [Montagne *et al.*, 2003].

The propionate goes probably to the liver, and the acetate with methanol and hydrogen through the liver to the muscles and lungs, respectively [Stephen, 1994]. Todesco *et al.* [1991] presented that when propionate was incorporated into bread, it reduced post-prandial blood glucose response in healthy subjects by 38%. Studies carried out on rat liver cells imply that physiological concentrations of propionate may attenuate hepatic cholesterol synthesis [Anderson & Bridges, 1984]. Venter *et al.* [1990] demonstrated a beneficial effect of propionate on man serum cholesterol, dietary propionate increased the high-density lipoprotein cholesterol fraction (HDL). Acetate is always found in human venous blood with fasting levels of around 50 μmol/L, rising to 100–300 μmol/L after meals containing fermentable carbohydrate. It is cleared from the blood with a half-life of only a few minutes, and is a metabolized primarily by skeletal and cardiac muscle and the brain. Patients-ileostomists have low blood-acetate levels ($<20\,\mu$mol/L). Acetate inhibits fatty acid oxidation in man and free fatty acid level is observed to fall when acetate or alcohol, which is an immediate precursor of acetate, is administered [Cummings & Macfarlane, 1997].

The 24-h *in vitro* fermentation of the ileal effluents, obtained after diet supplementation with 30 g of raw green banana flour (RS_2), showed that the pH lowers significantly and the concentration of total short chain fatty acids increases, especially of acetate and butyrate [Langkilde *et al.*, 2002]. Additionally, the molar ratio of butyrate was significantly higher whereas the molar ratio of propionate was lower in the fermented ileal effluents in this experiment.

Among SCFA, butyrate has been shown to play a key role as an energy for metabolism and proliferation of colonocytes. It performes two functions: most of the butyrate is a substarte for aerobic adenosine triphosphate (ATP) production; and it acts specifically like a signal metabolite, stimulating cell migration and proliferation. That is why butyrate is so important for the colonic mucosa and in providing protection against colon cancer and colitis [Christl *et al.*, 1996; Csordas, 1996; Velazquez *et al.*, 1996; Archer *et al.*, 1998; Jacobasch *et al.*, 1999].

Butyrate is formed from two molecules of acetyl coenzyme A (CoA), yielding acetoacetyl-CoA, which is then converted, *via* the intermediates L(+)-β hydroxybutyryl CoA and crotonyl-CoA, to butyryl CoA [Pryde *et al.*, 2002]. Thereafter, butyryl-CoA may yield butyrate *via* butyrate kinase, as in some strains of ruminal *Butyrivibrio fibrisolvens* or *via* butyryl-CoA:acetate-CoA transferase. In the latter reaction butyryl-CoA is exchanged with exogenously-derived acetate to yield acetyl-CoA and butyrate [Diez-Gonzales *et al.*, 1999]. Diez-Gonzales *et al.* [1999] suggested the existence of two distinct metabolic types, differing in acetate utilization and lactate formation, among butyrate-producing *Butyrivibrio fibrisolvens* strains from the rumen, apparently correlating with the possession of either butyrate kinase or butyryl-CoA:acetate-CoA transferase. Barcenilla *et al.* [2000] isolated a range of butyrate-producing strains from human faeces. Most (>80%) of the strains that pro-

duced high concentrations (>10 mmol/L) of butyrate in batch culture *in vitro* have now been identified by 16S rDNA sequencing and phenotypic tests as species of the gram-positive anaerobes *Roseburia* [Duncan *et al.*, 2002] and *Faecalibacterium* (previously *Fusobacterium*).

Velázquez *et al.* [1996] showed the paradoxical effects of butyrate on normal and neoplastic colonocyte proliferation and differentation. They observed that 10 mmol/L of butyrate induces proliferation of the colonic crypt base (undifferentiated rapidly proliferating normal cells) while inhibiting proliferation at the crypt surface (differentiated normal cells with low proliferation rates) in the surgically-isolated normal rat colon *in vivo*. Butyrate may act as a cofactor to proteins in the transduction pathway or to the transcription regulatory proteins, thereby specifically stimulating or inhibiting certain cellular processes. It has been suggested in scientific literature that butyrate may not only protect against the initiation and development of large bowel cancer [Avivi-Green *et al.*, 2000] but perhaps also breast [Heerdt *et al.*, 1999] and prostate cancer [Ellerhorst *et al.*, 1999].

Studies on gut metabolism of patients and experimental animal models suffering from gut inflammation have shown that a sufficient and sustained level of SCFA may be essential for the maintenance of a healthy gut. Patients with inflammatory bowel disease and with colon cancer typically have low levels of butyrate in the gut and have a low rate of butyrate oxidation by the mucosa [Brouns *et al.*, 2002]. It could be related to a poor supply of fermentable substrate and non-balanced intestinal microflora, in which sulfate reducing bacteria are present in too large quantities. These bacteria produce increased luminal concentration of mercaptans, sulfides and sulfites. Mercaptans are known to inhibit the uptake of butyrate by the colon cells [Stein *et al.*, 1995]. In a situation of poor supply of fermentable substrate and accordingly low levels of SCFA production, an appropriate supply of butyrate to the colonocytes is further diminished by the action of mercaptans. This may lead to a short-age of energy for optimal functioning and to breakdown of adenine nucleotides along with the formation of free radicals through the xanthine oxidase reaction. Both of them will promote the inflammation and ultimately the necrosis process in the intestine [Jacobasch *et al.*, 1999].

Several colonic bacterial groups have the metabolic capacity to ferment starch *in vitro* and the studies showed that starch fermentation by faecal inocula appeared to favour butyrate production [Weaver *et al.*, 1992]. Soral-Śmietana *et al.* [2001a] presented the results of the 24-h fermentation of medium containing experimental starch preparations with high level of resistant starch, originating from the wheat and potato starches, inoculated with faecal microflora of rats. Bacterial fermentation of starch preparations resulted in enhancement of acidifying activity, as the pH was decreased more than about 0.3 comparing with control. During 24-h of fermentation in medium with these substrates, the different ratio of the main SCFA, the acetic: propionic: butyric, was noticed. Compared to the control the decreasing share of acetic acid and increasing the concentration of propionic and butyric acids after 6-, 12-, and 24-h fermentation of wheat and potato substrates were observed (Table 1) [Soral-Śmietana, 2000].

Nutritionists recommended an increased consumption of non-digestible polysaccharides in the Western diet, as for many people this intake remains low. An intake of around 18 g/d non-starch polysaccharides (NSP) and 20 g/d of resistant starch (RS) appears necessary for beneficial effects to the bowel [Cummings & Englyst, 1995]. However the intake of RS in some countries analysed occasionally indicated almost half this dose or lower [Dysseler & Hoffem, 1995; Baghurst *et al.*, 1996; Soral-Śmietana & Wronkowska, 1999; Soral-Śmietana, 2000]. Martin *et al.* [2000] demonstrated that the consumption of diets containing various types of RS induces different patterns of appearance of SCFA in the portal blood. Among the three RS sources tested, only the raw potato starch led to a significant appearance of butyrate in the portal blood. It seems that RS fermented in the most distal part of the colon could be more beneficial for a healthy colon, since the uptake of butyrate by the colonocytes is much higher. But raw potato starch

TABLE 1. Changes of proportion between individual short-chain fatty acids during *in vitro* fermentation of wheat and potato resistant starch preparations with rat microflora [Soral-Śmietana, 2000].

Substrate for fermentation	Short-chain fatty acids (%)				
	Acetic	Propionic	Butyric	Isovaleric	Valeric
			after 6 h of fermentation		
control (no RS-content)	53.4	29.7	11.8	5.0	trace
with RS-preparation:					
wheat	43.0	32.2	18.4	6.4	trace
potato	40.4	32.1	21.6	5.9	trace
			after 12 h of fermentation		
control (no RS-content)	46.0	29.9	17.3	6.7	trace
with RS-preparation:					
wheat	40.7	31.3	21.4	6.5	trace
potato	32.9	24.4	20.8	11.8	10.0
			after 24 h of fermentation		
control (no RS-content)	41.8	29.5	20.5	8.0	trace
with RS-preparation:					
wheat	39.3	28.5	23.7	8.4	trace
potato	24.7	20.9	21.2	14.2	19.0

(RS_2) could produce a large amount of butyrate without any benefit to the colonic epithelium. In order to prevent or to treat the colon disease, it would appear to be necessary to select the RS sources not only with respect to their potential butyrate production, but also to the *in vivo* metabolism of butyrate by the colonic mucosa. Young *et al.* [1996] presented that rats fed a diet containing 20% of raw potato starch had longer and more frequent tumours than rats consuming a basic diet or the same diet enriched with wheat bran.

CHOLESTEROL AND BILE ACIDS

The liver plays a key role in the regulation of cholesterol homeostasis in humans. Circulating LDL originates from very low-density lipoprotein (VLDL) produced in the liver and is mainly taken up and degraded by this organ. A large portion of LDL-cholesterol is then converted to bile acids and removed from the body by biliary excretion [Tatidis *et al.*, 2001]. During ileostomy study high-resistant-starch banana flour (RS_2) in the diet of human subjects did not affect cholesterol, total sterols or total bile acids [Langkilde *et al.*, 2002]. There was, however, a difference of the separate bile acids, *i.e.* chenodeoxycholic acid was higher in ileal excretion and no significant change of cholic acid as was observed as the effect of diet supplementation with 30 g of raw green banana flour (RS_2).

The Vanhoof & De Schrijver [1998] investigated whether the addition of 6% of commercial corn resistant starch type RS_2 (10.8% Hylon VII) or RS_3 (14.6% Novelose) to conventional diets of rats had any effect on cholesterol metabolism and excretion of bile acids. No significant effect of enzyme-resistant starch feeding on plasma and liver cholesterol concentrations was found. However consumption of RS type 2 or type 3 influenced liver cholesterol metabolism, resulting in a biologically-important decrease in esterified and total cholesterol of 24% and 22%, respectively. The RS type 3 caused faecal excretion of a biologically-important neutral steroid, coprostanol, in normocholesterolaemic (25%) and hypercholesterolaemic (40%) rats. The Vanhoof & De Schrijver [1998] discussed that while considering the effect of RS consumption in humans where dietary fibre intake is very low, the overlapping of fibre and RS must be taken into account. Consequently, RS intake might influence cholesterol metabolism in human taking a low-fibre diet. Although no significant effect of RS feeding on cholesterol metabolism in rats, may not reflect its effect in humans, particularly as rats have a different bile acid profile than humans [Heuman, 1989]. The cholesterol lowering effect in rats has not been reported in human studies with normolipidemic subjects [Heijnen *et al.*, 1996]. However, significantly lowered plasma cholesterol and triacylglycerols concentrations, resulting from the intake of a diet enriched with 20% of raw potato starch or high amylose corn starch compared with fibre-free diet, were found in rats [Lopez *et al.*, 2001].

The effect of potato resistant starch (RS_2) and cholestyramine (CHY) on cholesterol metabolism were tested in the rats [Younes *et al.*, 1995] and in guinea pigs [Fernendez *et al.*, 2000], because they present many similarities to humans in plasma lipoprotein distribution and responses to diets. The guinea pigs fed on RS and CHY exhibit lower plasma LDL cholesterol concentrations than those treated with cellulose, whereas their plasma triacylglycerol levels did not differ. In contrast to cholestyramine tested in rats [Younes *et al.*, 1995], the RS_2 (25% raw potato starch) depressed the concentration of triacylglycerols in the triacylglycerol-rich lipoprotein fraction. Any noticeable synergy was postulated between the effects of RS and CHY when both these were presents in the rats' diet. The potato RS_2 treatment in the guinea pigs caused a significant reduction in plasma LDL cholesterol, without affecting plasma TAG, however it modified TAG metabolism (lower hepatic TAG concentrations and fewer number of TAG molecules in LDL) [Fernendez *et al.*, 2000]. It have been discussed that potato RS_2 could possibly have similar mechanism of action as dietary soluble fibre in the intestinal lumen. Two major mechanisms have been observed in the guinea pig [Fernendez *et al.*, 2000]: (1) decreases in cholesterol absorption, and (2) interruption of the enterohepatic circulation of bile acids. However the investigated RS_2 did not up-regulate cholesterol 7α – hydroxylase activity, since a decrease in hepatic cholesterol was observed. The Fernendez *et al.* [2000] supposed that this RS decreased the absorption and delivery of cholesterol to the liver by the chylomicron remnant. Depending on the RS intake related to the decreased dietary cholesterol absorption, it could be due to the viscosity of RS or to its gel formation properties that might disrupt micelle formation or increase the unstirred water layer.

The plasma levels of 7α-hydroxy-4-cholesten-3-one have been shown to reflect the activity of hepatic cholesterol 7α-hydroxylase, which catalyses the rate-limiting reaction of bile acid synthesis and total bile acid production measured by isotope-dilution techniques [Sauter *et al.*, 1996]. Levels of cholesterol precursor 7-dehydrocholesterol reflect the hepatic activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the rate-limiting enzyme for cholesterol synthesis. This marker is the immediate precursor of cholesterol [Tatidis *et al.*, 2001]. Degradation of cholesterol to bile acids is one of the major pathways by which cholesterol is eliminated from the body [Goel *et al.*, 1998].

The primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are derived *via* two different metabolic pathways from cholesterol in the liver and are secreted in the bile mainly as glycine or taurine conjugates. Under physiological conditions, the rate-limiting enzymes in the synthesis of CA and CDCA are cholesterol 7α-hydroxylase (CYP7a1) and sterol 12α-hydroxylase (CYP8b1). The activity of CYP8b1 may determine the ratio of CA to CDCA, and hence, the hydrophobicity of the circulating bile acid pool [Debruyne *et al.*, 2001]. Approximately 85% of the bile acids delivered to the duodenum are absorbed back into blood within the ileum but about 15–20% of the secreted bile acids reach the colon and are metabolized in the large bowel by the anaerobic bacterial flora. First, deconjugation takes place and the amino acid molecule on the carboxyl group is removed. Secondly, secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA), are formed from conjugates forms of cholic acid and chenodeoxycholic acid. Deoxycholic acid is partly absorbed in the colon and enters the enterohepatic circulation where it is conjugated in the liver and secreted in bile; lithocholic acid is almost insoluble and hardly reabsorbable.

DCA and LCA are excreted in the stool and make up to 95% of the total amount of excreted bile acids. In the stool they are bound to dietary and bacterial residues [Nagengast *et al.*, 1995]. Deoxycholic acid and lithocholic acid, the main faecal bile acids, are suspected to be the forms of bile acids that are implicated in colorectal carcinogenesis [Debruyne *et al.*, 2001]. The results of *in vitro* sorption of bile acids by starch preparations (about 60% content of RS) obtained after the physico-biochemical process of native wheat and potato starches [Soral-Śmietana *et al.*, 2001a] or native pea starch [Soral-Śmietana & Wronkowska, 2000] were presented. That investigation was based on cholic, deoxycholic and taurocholic acids. The wheat and potato preparations indicated a higher sorption of bile acids in comparison to native starches. However, especially significant sorption of deoxycholic acid was obtained for pea preparation.

Hori *et al.* [1998] showed the effect of dietary deoxycholic acid (DCA) and cholesterol (CHL) on the faecal composition and concentrations of neutral and acid steroids. Rats fed a diet supplemented with 0.15% DCA and 1% CHL had a fivefold and tenfold increase in faecal bile acids and neutral steroid levels, respectively, when compared to rats fed a control diet. It was associated with a higher proliferation of colonic epithelial cells, 2 and 1.5-times higher than those of the control diet groups, respectively.

The primary and secondary bile acids excretion by rats faecal was observed after the addition of 6 % corn resistant starch type RS_2 (10.8% Hylon VII) or RS_3 (14.6% Novelose) to conventional diets [Vanhoof & De Schrijver, 1998]. The hypercholesterolaemic rats, however, had higher faecal excretion of all individual bile acids as well as total bile acids compared with the normocholesterolaemic group. It has been shown that RS has the capacity to form inclusion complexes with bile acids due to its helical structure [Abadie *et al.*, 1994]. Not every type of RS or its origin influenced the extent of the bile acids binding in the same rate as it was observed at *in vitro* estimation [Soral-Śmietana *et al.*, 1999; Wronkowska & Soral-Śmietana, 2000; Soral-Śmietana & Wronkowska, 2000]. The studies of van Munster *et al.* [1993, 1994] showed a decrease of soluble deoxycholic, lithocholic and chenodeoxycholic acids in the aqueous phase of stool in humans consuming a daily dose of 3 × 15 g of RS_2 (native uncooked high amylose corn starch).

GUT MICROFLORA – BIOMASS, BACTERIAL GROWTH, CHANGES OF FAECAL ANAEROBIC BACTERIA

The human gastrointestinal tract consists of the mouth, oral cavity, esophagus, stomach, small intestine and colon. The large intestine starts at the ileocaecal junction, with anatomically distinct regions of this organ being the caecum, ascending colon, transverse colon, descending colon and sigmoid colon. Biological functions of the large intestine include the absorption and secretion of certain electrolytes and water, as well as storage and excretion of waste materials. In the past decade much attention has been put on the gut microbiota. The human large intestine can be described as a complex microbial ecosystem. The principal role of the intestinal microflora is the salvage of energy from carbohydrates not digested in the upper gut, through fermentation. The major substrates for fermentation include starch that enters the colon (resistant starch), as well as non-starch polysaccharides, *e.g.* cellulose, hemicelluloses, pectins and gums. Other carbohydrate sources available for fermentation are non-digestible oligosaccharides [Tomomatsu, 1994], various sugars and sugar alcohols [Cummings *et al.*, 1997].

The gastrointestinal tract of new born infants is inoculated by the mother's vaginal and faecal flora during birth. There is a predominance of facultatively anaerobic strains such as *Escherichia coli* or enterococci. These bacteria may create a highly reduced environment that then allows the growth of strict anaerobes, but there are differences in the composition of the gut microbiota in response to the infant's feeding [Gibson & Roberfroid, 1995]. The faecal flora of breast-fed infants is dominated by populations of bifidobacteria, with only about 1% of enterobacteria. But formula-fed infants have more complex microbiota, with bifidobacteria, bacteroides, clostridia and streptococci all prevalent [Yoshiota *et al.*, 1991].

In the human gastrointestinal tract, principally in the colon, at least 500 different microbial species exist, on a quantitative basis around 10 to 20 genera probably predominate. Examples of these include *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Lactobacillus*, *Fusobacterium*, *Peptococcus*, *Peptostreptococcus*, *Escherichi* and *Veillonella* [Gibson, 2002]. Most human large intestinal microorganisms have a strictly anaerobic metabolism. The numerically-predominant anaerobes were Gram-negative rods of the genus *Bacteroides*. They can constitute up to 30% of the total faecal flora. Other numerically-predominant groups are Gram-positive rods of bifidobacteria, eubacteria, clostridia, lactobacilli [Salminen *et al.*, 1998].

Almost 40–55% of colonic solids (faeces) is bacterial mass. Toxic metabolites in 300 g of wet faeces include 186 mg of ammonia, 1.4 mg of phenol, 12.2 mg of p-cresol, 8.5 mg of indole, and 3.3 mg of skatole [Tomomatsu, 1994]. The toxic metabolites are formed by detrimental bacterial enzymes. Enzyme formation depends on the bacteria and the gastrointestinal ecology. The bacterial enzymes commonly assayed include β-glucuronidase, β-glucosidase, azoreductase, nitroreductase, nitrate reductase and others. The substrates for these enzymes and the functional and health implications of their products have been reviewed. For example, bacterial β-glucuronidase in the colon is able to release carcinogens from hepatically-derived glucuronic acid conjugates and is a critical factor in the enterohepatic circulation of drugs and other foreign compounds [Salminen *et al.*, 1998].

An analysis of human faeces shows that bifidobacteria form one of the predominant culturable bacterial groups in the human colon where their number often reach the level of 10^9–10^{10} cfu g^{-1} [Alander *et al.*, 2001]. Bifidobacterial numbers in the human gut tend to decrease with age. Bifidobacteria prevent the growth of exogenous pathogenic microbes and the excessive growth of indigenous detrimental microflora through the production of short-chain fatty acids, mainly acetic acid and lactic acid, and demonstrate an ability to produce some antibiotic materials [Tomomatsu, 1994; Gibson & Roberfroid, 1995; Lee *et al.*, 1999].

An increase in the number and activity of bifidobacteria (and lactobacilli) in the colon is desirable. The addition of live cultures to foodstuffs such as fermented milk products [Crittenden *et al.*, 2001] is often referred to as "probiotic".

Probiotics have been variously defined largely on the basis of their initial use in animal feeds. For the purposes of human nutrition Salminen *et al.* [1998] suggested that a probiotic is a live microbial food ingredient that is beneficial to health. The probiotic microorganisms need to colonise the colon and, preferably, become active. Before they come to the colon they need to adhere to the intestinal epithelium. The bacteria would need to compete for nutrients and ecological sites of colonisation with a previously established microbial flora. Because of some limitations of probiotics the use of prebiotic is more comfortable. According to Gibson & Roberfroid [1995], a prebiotic is a non-digestible food ingredients that beneficially affects the host by selectively stimulating growth and/or activity of one or a limited number of bacteria in the colon, that have the potential to improve host health. Non-digestible oligosaccharides that are fermented in the colon, are now the most popular group of prebiotics [Tomomatsu, 1994; Alander *et al.*, 2001] but other carbohydrates appeared to have prebiotic potential. Alginates, which are used widely in the food industry, and RS may also be useful as prebiotic [Bird *et al.*, 2000].

Brown *et al.* [1997] demonstrated that the total faecal excretion of bifidobacteria was higher when pigs were fed live *Bifidobacterium longum* with the high amylose corn starch. Studies *in vitro* have shown that bifidobacteria can adhere to chemically-modified starches [Brown *et al.*, 1998]. This adhesion varied by strain and did not extend to *Lactobacillus casei*. These data show that RS_2 and RS_4 have the potential to act as prebiotics. Other studies support the prebiotic action of RS and that the effect varies with type of starch in the diet. Kleessen *et al.* [1997] showed an increase in lactobacilli and *Bifidobacterium sp*. in the caecum of rats fed a diet containing raw and modified potato starch for 5 months.

EPITHELIAL CELL PHYSIOLOGY AND CELL BIOLOGY

Colorectal cancer is the fourth most common cause of cancer-related mortality in the world. Within Europe, North America, Australia and New Zealand, it is the second most common cancer after lung or breast cancers [Boyle & Langman, 2000]. Evidence suggests that diet plays a significant role in the aetiology of colorectal cancer. Colonic micro-architecture is characterized by crypts, which are approximately fifty cells in depth. The normal structure and replicative dynamics of the crypts ensure that both stem cells and immediate daughter cells replicate in the lowest region. When the immediate daughter cells divide and migrate they give rise to all the cells that line the crypt. Eventually these cells will reach the surface by which stage they are fully differentiated columnar epithelial cells, covered with microvilli, intimately connected *via* numerous tight junctions and involved in water and electrolyte transport [Gill & Rowland, 2002]. There are some theories about the first mutagenic agents. Potter [1999] used the nature of the micro-architecture to argue that the first mutagenic event occurring to a progenitor cell would have to be a blood-borne rather than a luminal agent. Others authors presented that a luminal agent could provide the first mutation and then directly or indirectly affect cell in crypt and lead to the formation of a polyp [Bruce *et al.*, 2000]. The potential role of luminal factors in the development of colonic tumours has led to the theory that the large bacterial population in the colon is involved in the formation of carcinogens, tumour promoters and anticarcinogens in the gut.

Colon cancer arises out of perturbation of the normally-ordered and balanced proliferation and deletion mechanisms of the cell crypt. This results in the hyperproliferation and a shift in the proliferation zone from a restricted band to the entire crypt [Wilson *et al.*, 1990]. Colon mucosal cells are under constant, but low, genotoxic stress. Such damage is normally repaired, but any factors influencing the integrity of the repairs process are important in determining the risk of cancer. DNA mismatch repair genes, if inactivated, tend to result in the colorectal mucosal cells accumulating additional mutations at a higher rate, potentially enhancing their capacity for malignant transformation [Boland, 1996].

The scientists search for dietary components that modulate the gut microflora and its activities and thus may influence colorectal cancer, *e.g.* probiotics, prebiotics and fibre. More than 500 dietary compounds have been identified as potential modifiers of cancer. The hypothesis that fibre might decrease cancer risk, especially in the colon, has been a topic of discussion for well over a quarter of a century. Not all fibre sources are equivalent in their ability to alert cancer risk [Milner, 2002]. The fermentation of fibre in the colon has many other biological and cellular effects, which include changes in the process of crypt fission [McCullough *et al.*, 1998], which may be an essential event in the initiation and development of colorectal polyps [Wasan *et al.*, 1998]. Some observation suggest that resistant starch, like soluble fibre, has a positive impact on colonic health by increasing the crypt cell production rate, or decreasing the colonic epithelial atrophy in comparison with fibre-free diets. It has been indicated that resistant starch, like guar, a soluble fibre, influences tumorigenesis [Haralampu, 2000].

The relationship between diet, microflora and colorectal cancer is complex and intimate. Substances entering the colon from ileum and the resident microflora are major determinants of colonic physiology. These, together with the innate biology of the colon, are pertinent to the initiation and promotion of colorectal cancer [Gill & Rowland, 2002].

NEW ASPECT OF DIETARY FIBRE DEFINITION

The presented results of the last twenty years investigations into resistant starch, a frequent diet component, show many distinctive, physiological values of this starch fraction, often in comparison with dietary fibre. A special attention is paid to its function within the autochthonous microflora of the human or animal large intestine and its preventive activity against non-infectious or chronic diseases.

Over the past decade, non-digestible oligosaccharides (NDO) and resistant starch (RS) were recognized mainly as a separate components or sometimes as dietary fibre components, based on their physiological behaviour. Recently the dietary fibre definition was taken up under the oversight of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes initially by American Association of Cereal Chemists [AACC Report, 2001] and then by the (US) Food and Nutrition Board of the Institute of Health [Anonymous, 2001]. The refined definitions of dietary fibre are proposed as follows.

American Association of Cereal Chemists [AACC Report, 2001] definition: "Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation."

Food and Nutrition Board of the Institute of Health [Anonymous, 2001] definition: "Dietary Fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. Added Fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in human. Total Fiber is the sum of Dietary Fiber and Added Fiber."

The concept of both of these definitions is based on the most-widely-accepted physiological area. These functional definitions for dietary fibre are necessary for measuring not only the quality of a diet, consisting in eating plenty of high-fibre foods including whole grains, fruits and vegetables, but also for measuring the finite portion of food or food supply for the research, regulation and labelling purposes. The resistant starch is considered to be a part of dietary fibre according to a newly proposed definition. Asp [2001] commented: "The definition and analysis of dietary fibre are intimately related. Analysis methods have to be developed in accordance with the conceptual definitions, but in practice, compromises must be accepted due to constraints of cost and time. All types of dietary fibre components can be separated at the different levels of complexity and determined separately for the research purposes, though short-hand methods are needed for labelling and control purposes."

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FINAL REPORT

Title of the research ordered project:
THE METHODOLOGICAL BASES OF THE EVALUATION OF THE QUALITY AND SAFETY OF THE NEW GENERATION FOOD (PBZ-KBN-020/P06/1999).

Title of the individual project:
Amylase-resistant starch preparation – searching for a food components with specific biological activity (task 03).

Institution:
Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Division of Food Science; Department of Functional Properties of Food; Olsztyn, Poland.

Leader:
Prof. dr hab. Maria Soral-Śmietana

Co-workers:
Prof. dr hab. Maria Bielecka, Dr inż. Małgorzata Wronkowska, Dr inż. Elżbieta Biedrzycka, Doc. dr hab. Grażyna Lewandowicz, Doc. dr hab. Ryszard Amarowicz.

Key words:
Native starches: wheat, potato, pea, physical modification of starches, amylase-resistant starch preparations, *Bifidobacterium* strains, stimulation of bifidobacteria growth, acidifying activity.

SYNTHESIS OF RESULTS

The aim of this research was searching for the biological activity of starches studied by the microbiological testing of *Bifidobacterium* strains acc. to *in vitro* method based on the three origins of native starches and their starch preparations with different contents of the resistant starch fraction.

Starches: wheat, potato, pea were the reference and initial material to obtain starch modified preparations in the double way: (I) – by iterated syneresis of starch gel; (II) – by the combination of the physical and hydrothermal processes using thermostable α-amylase. Both kinds of preparations contained the amylase-resistant starch fraction: (I) – low or medium content (8–19%); II – high content (64–73%). The investigations of crystal structure of the preparations (I) showed the B-type of X–ray pattern. The preparations (II) had crystal structure but non-typical, unlike to the type of starch structure before processing. The modification by iterated syneresis of gel caused another mechanism of gelatinisation provoked with lower gelatinisation temperature. Based on differential scanning calorimetry (DSC), the melting temperature of the preparations (I) crystallite was typical of retrograded starches, but the enthalpy was higher. However, for preparations (II), the temperature of phase transition Tp 128 to 140°C was observed, while Tc reached 143–156°C, but the enthalpy of wheat and pea preparations was higher in comparison with starch before modification, and contrary tendency for potato preparation was noticed. On the other hand, the isotherms sorption of water vapour of the native starches and these preparations (II), according to UPAC classification, showed strong II-type and week III-type interactions.

The infrared spectra (FTIR) prove that the changes of starch structure after modification by syneresis are noted not only in the region characteristic for polysaccharides, 1400–800 cm^{-1}, but also in the range to 400 cm^{-1}, as the phenomenon called "finger-print". It appeared especially in modified wheat starch. Unexpectedly, the lowest level of the resistant starch fraction was observed in native wheat starch analysed, which probably results from the biological diversification within the wheat species.

The resistant starch fraction of the obtained modified preparations was termed as follows: (I) – RS_3-type, (II) – RS_3/RS_4-type and native starches as RS_2-type.

The microbiological studies (*in vitro*) were carried out to determine the intensity of the utilisation of native starches and their preparations as the substrates for *Bifidobacterium* strains as a carbon and energy source. The *Bifidobacterium* strains, existing in the infant intestine, the large bowel of people and animals and with good abilities to grow on the medium with the native starches instead of glucose, were selected out of 75 *Bifidobacterium* strains. Three of these strains: *B. breve* KM14, *B. pseudolongum* KS19 and *B. animalis* KS20a1, utilising these starches for the stimulation of the population growth and showed ability to lowering pH during 24-h anaerobic fermentation, were investigated. The short-chain fatty acids: acetic, butyric, lactic, propionic were the fermentative metabolites analysed *in vitro* under anaerobic conditions by *Bifidobacterium* strains, but acetic acid or lactic acid were found to dominate, which depended on the origin of starch and bifidobacteria strain. The range of the metabolisation of the resistant starch fraction present in the starches (native and their preparations) was various, but the majority of the analysed material was utilised in 20–77%, especially pea and potato starch were distinguishing as their preparations were fermented by *B. breve* KN14 and *B. animalis* KS20a1 strains. Therefore, the bifidogenic properties of the resistant starch fraction determined during these studies showed that the way and degree of the polysaccharide utilisation by microflora is dependent on the botanical origin of polysaccharide substrate and the saccharolytic activity of *Bifidobacterium* strain.

Perspective determination of a quality attribute of a food product containing a polysaccharide-component

(natural or technologically-modified starch), could be estimated in starch products as:

An attribute of potential biological activity of resistant starch fraction.

This would aim at determining the intensity of utilisation of starch/amylose-resistant fraction – an energetic substrate for the growth of bifidobacterial strains – compared with glucose as a standard source of carbon and energy. Based on a conventional scale, where the growth results on glucose as a microbiological substrate of beneficial bacterial flora would constitute a reference point = "10", whereas the range of selective metabilisation of starch substrates could be determined based on the growth effect of 2 *Bifidobacterium* strains investigated in this research, *e.g. B. breve* KN14 and *B. animalis* KS20a1 well utilising the examined starches and their resistant starch fractions. Still, the metabolic assumption in the pool of volatile fatty acids (VFA) formed should be a potential quantitative advantage of acetic acid over lactic acid, both obtained during anaerobic fermentation of polysaccharides constituents.