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Resistant Dextrins as Prebiotic

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<http://dx.doi.org/10.5772/51573>

1. Introduction

In current days, the way each of human beings live indicates his or her health in the future time. Many factors determine the risk of illnesses, or reversibly, the possibility of being healthy. Being physically active and consumption of appropriate diet are examples of daily routines that may influence the condition of an organism. Lack of physical activity, particularly if associated with over consumption, increases the risk of development of nutrition related chronic diseases, such as obesity, hypertension, cardiovascular diseases, osteoporosis, type II diabetes, and several cancers. Over the last decade, drastic changes have taken place in the image and assessment of the importance of the daily diet. Foods are no longer judged in terms of taste and immediate nutritional needs, but also in terms of their ability to improve the health and well-being of consumers. The role of diet in human health has led to the recent development of the so-called functional food concept. A functional food is dietary ingredient, that has cellular or physiological effects above the normal nutritional value. Functional food can contain probiotics and/or prebiotics.

2. The prebiotic concept

A number of different strategies can be applied to modify microbial intestinal populations. Antibiotics can be effective in eliminating pathogenic organisms within the intestinal microbiota. However, they carry the risk of side effects and cannot be routinely used for longer periods or prophylactically [17, 33].

The consumption of probiotics aims to directly supplement the intestinal microbiota with live beneficial organisms. Lactobacilli and bifidobacteria are numerically common members of the human intestinal microbiota, and are nonpathogenic, nonputrefactive, nontoxigenic, saccharolytic organisms that appear from available knowledge to provide little opportunity for deleterious activity in the intestinal tract. As such, they are reasonable candidates to target in terms of restoring a favorable balance of intestinal species [18, 85].

Prebiotics represent a third strategy to manipulate the intestinal microbiota. Rather than supplying an exogenous source of live bacteria, prebiotics are nondigestible food ingredients that selectively stimulate the proliferation and/or activity of desirable bacterial populations already resident in the consumer's intestinal tract. Most prebiotics identified so far are nondigestible, fermentable carbohydrates. Intestinal populations of bifidobacteria, in particular, are stimulated to proliferate upon consumption of a range of prebiotics, increasing in numbers by as much as 10–100-fold in faeces [9, 17].

3. Advantages and disadvantages of the prebiotic

The prebiotic strategy offers a number of advantages over modifying the intestinal microbiota using probiotics or antibiotics.

Advantages over probiotics [17]:

- Stable in long shelf life foods and beverages;
- Heat and pH stable and can be used in a wide range of processed foods and beverages;
- Have physicochemical properties useful to food taste and texture;
- Resistant to acid, protease, and bile during intestinal passage;
- Stimulate organisms already resident in the host, and so avoid host/strain compatibilities, and the need to compete with an already established microbiota;
- Stimulate fermentative activity of the microbiota and health benefits from SCFA (short chain fatty acids);
- Lower intestinal pH and provide osmotic water retention in the gut.

Advantages over antibiotics [18]:

- Safe for long-term consumption and prophylactic approaches;
- Do not stimulate side effects such as antibiotic-associated diarrhea, sensitivity to UV radiation, or liver damage;
- Do not stimulate antimicrobial resistance genes;
- Not allergenic;

Disadvantages of prebiotics [17]:

- Unlike probiotics, overdose can cause intestinal bloating, pain, flatulence, or diarrhea.
- Not as potent as antibiotics in eliminating specific pathogens.
- May exacerbate side effects of simple sugar absorption during active diarrhea.

A consumed probiotic strain must compete with an already established microbiota, and in most cases they persist only transiently in the intestine. Individuals also harbor their own specific combination of species and unique strains within their intestinal bacteria suggesting that certain host–microbiota compatibilities exist. By targeting those strains that are already resident in the intestinal tract of an individual, the prebiotic strategy overcomes the need for probiotic bacteria to compete with intestinal bacteria that are well established in their niche [12, 17, 89, 103].

4. Definition of term “prebiotic”

The term prebiotics was first introduced in 1995 by Gibson and Roberfroid, defining “non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [33]. Definition brought up to date by Gibson specified prebiotic as “selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health” [32]. Current definition of prebiotics was suggested during ISAPP experts’ meeting in 2008 and it states that prebiotic is “dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” [24, 44].

Substances with prebiotic properties have to possess following properties [42, 74, 114, 116]:

- selectively stimulate growth and activity of chosen bacterial strains that have positive influence on health,
- lower pH of the bowel content,
- show positive for human spot action in the intestinal tract,
- be resistant to hydrolysis, action of intestinal tract enzymes and gastric acids,
- should not get soaked up in the upper part of the intestinal tract,
- should act as a selective substrate for one or for determined amount of beneficial species of microorganisms in the colon,
- should be stable in the process of food processing.

In order to evaluate and reason, if the given product is a prebiotic, the source of the substance should be given, as well as its purity, chemical composition and structure. It is very important to specify the carrier, concentration and amount in which it should be given to the host. Relating to the newest definition of the prebiotic, it was decided to type out three main criteria that have to be fulfilled by the substance in order to include it to the group of prebiotics [24].

1. Substance (component) – it is neither an organism, nor a medicine; substance that may be characterized chemically; in most cases this is a nutrient component.
2. Health benefits – calculable, exceeding any adverse effects.
3. Modulation – represents, that the presence of the substance and the preparatory, in which it is handled, changes the composition or activity of host microflora.

Prebiotics, similarly to other nutrient elements, have to fulfill certain safety parameters established in a given country. In the assessment of the final product following points should be taken into account [24, 39], (Figure 1):

1. If according to the legislation in the country, the history of safe use of the product in host is known (GRAS or its equivalent). If yes, the conductance of the following toxicological tests on animals and humans may not be necessary.
2. Safe, allowable norms for the consumption with minimal symptoms and adverse effects.

3. Product must not be infected and it should not contain any impurities.
4. Prebiotic cannot change the microflora in such a way, to cause a long-lasting harmful effect on host.

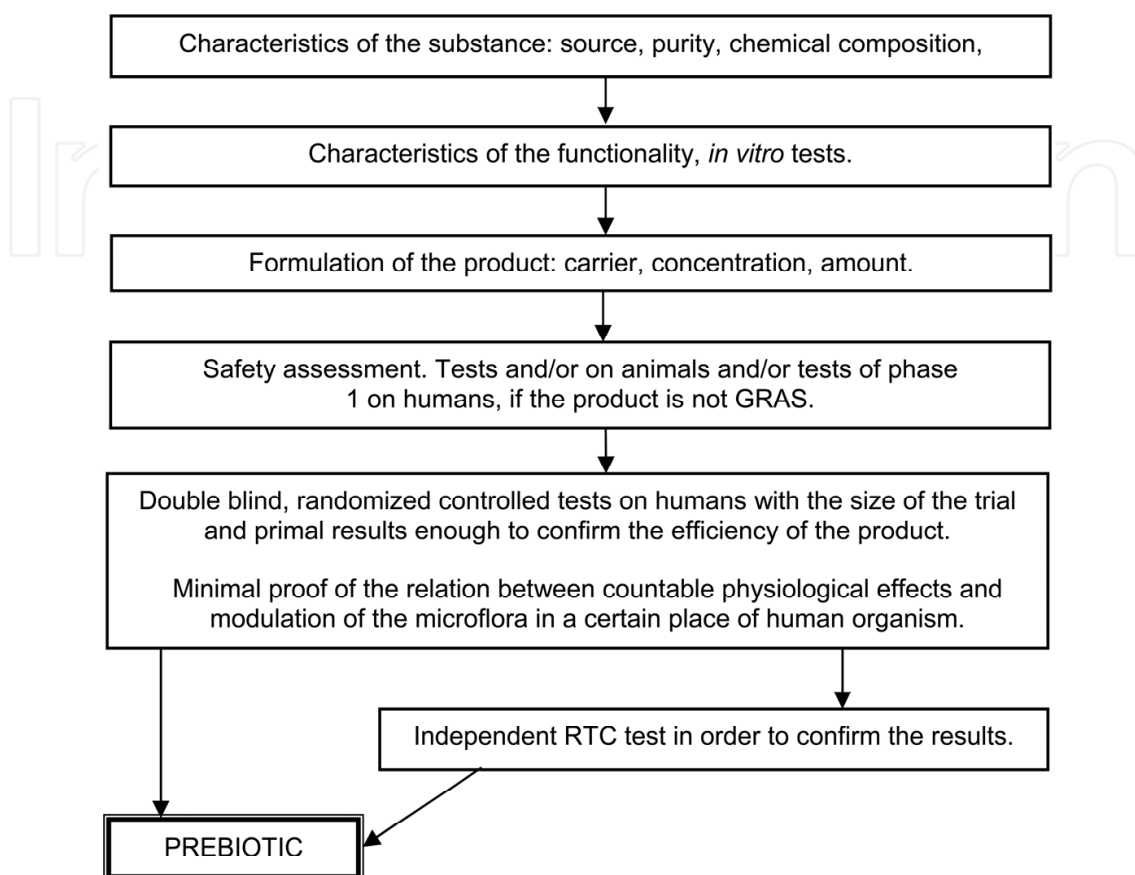


Figure 1. Guidelines to the assessment and proof of the action of prebiotics

5. Criteria of prebiotics classification

According to Wang [114] there are 5 most important basic criteria for classification of prebiotics (Figure 2). The first one assumes, that prebiotics are undigested in upper parts of intestinal tract and thus they are able to get to the large bowel where they can be fermented by potentially beneficial bacteria, which in the meantime meets the second criterion [61]. This fermentation may lead to increase in expression of short chain fatty acids, enlargement of faecal mass, some small reduction of large bowel pH, reduction of end nitrogen compounds and decrease of faecal enzymes, as well as general improvement of immunological system of host organism [17]. All these features contribute to improvement of consumer's health, which is the third criterion for prebiotics. The following one, that has to be fulfilled for the product to be recognized as prebiotic, is selective stimulation of growth of bacteria potentially thought to be connected with health improvement [32]. In order to assess the ability of prebiotic to selectively stimulate positive bacteria of species *Bifidobacterium* and *Lactobacillus*, so-called Prebiotic Index (PI) was introduced, and it can be calculated from the following formula [71]:

$$PI = (Bif/Total) - (Bac/Total) + (Lac/Total) - (Clos/Total)$$

Where:

Bif – *Bifidobacterium*

Bac – *Bacteroides*

Lac – *Lactobacillus*

Clos – *Clostridium*

Total – total bacteria

This PI allows to track the changes in the population in the given time in vitro conditions. At last, but not least, prebiotic should be able to survive the conditions in which the food would be stored, remain unchanged chemically and be accessible for bacteria metabolism [71].

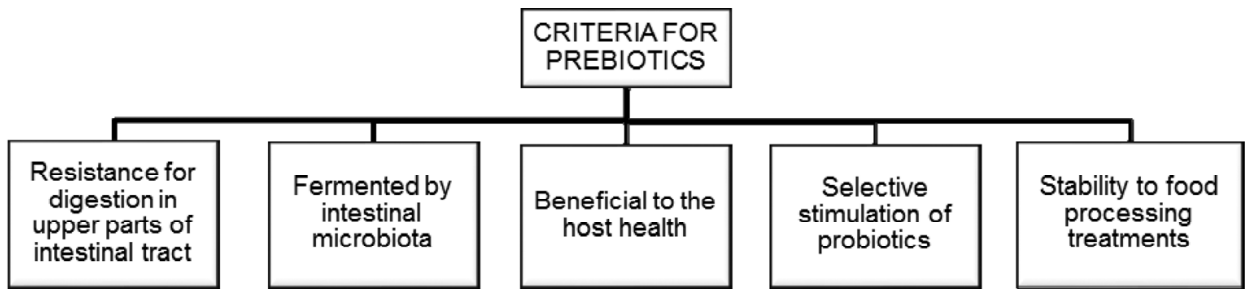


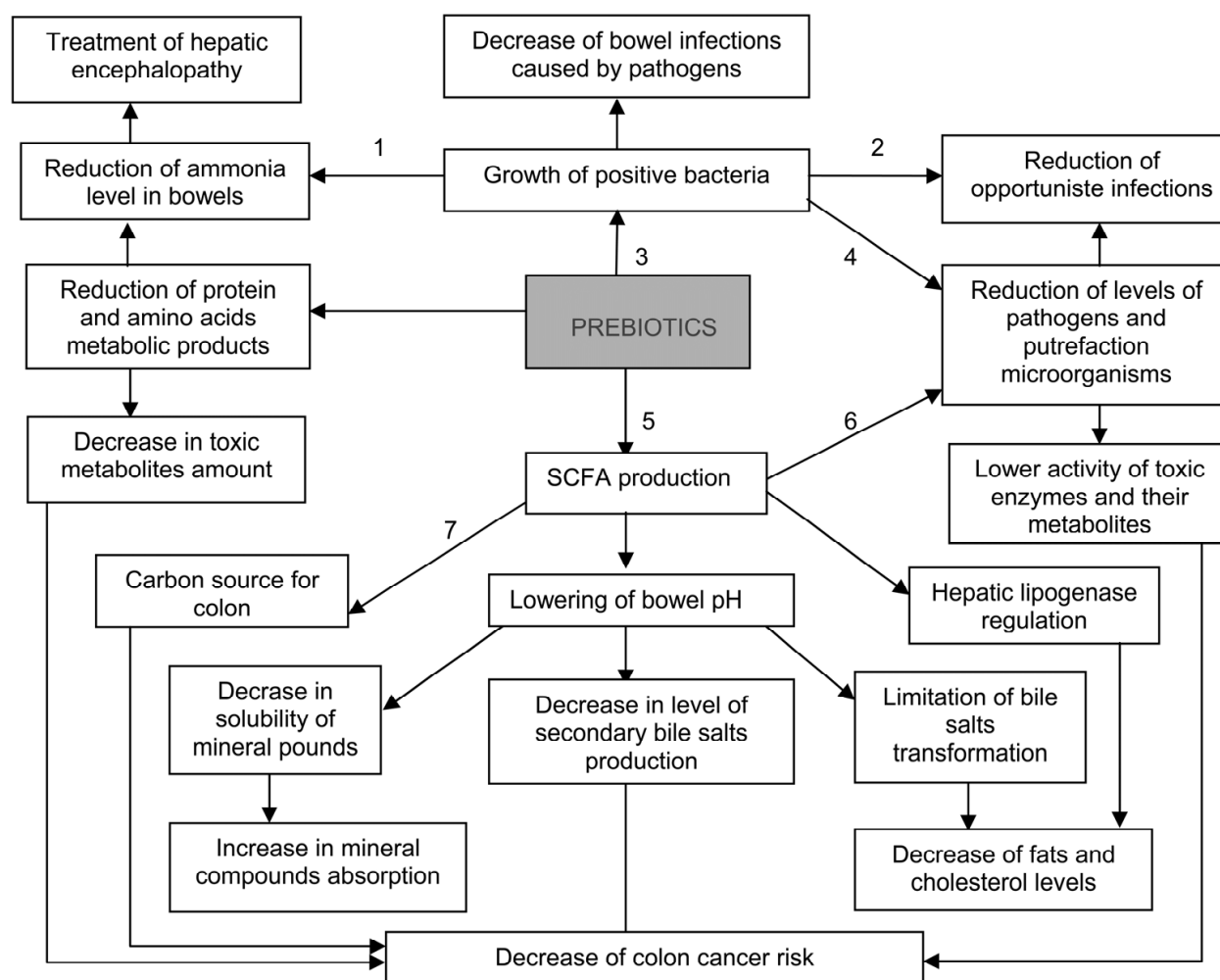
Figure 2. Criteria for prebiotics classification

6. Prebiotic mechanisms

The impact of prebiotics on the organism is indirect, because prebiotics do not do anything healthy for it, but they improve microorganisms that are beneficial [43]. The mechanism of how prebiotics influence the human health is presented on the Figure 3.

It is thought, that molecular structure of prebiotics is important taking into consideration the physiological effects, and that it determines which microorganisms are actually able to use that prebiotics. However, the way and progress of the stimulation of bacterial growth still remains unknown.

The most important function of prebiotic action is its influence on the microorganisms' growth and number in the large bowel [55, 90]. Going further, the tests have been conducted in order to investigate the potential ant pathogenic and anticancer action of prebiotics, their ability to decrease the presence of large bowel diseases [57]. A lot of different potential beneficial influences on human organisms are being sought, and those are, among the others: increase of the volume and improvement of stool moisture, lowering of the cholesterol level, decrease of the amount long chain fatty acids in bowels, decrease of pH in bowels, increase of mineral compounds absorption and raised short chain fatty acids production [1, 21, 90, 114], and the mechanism can be observed on the Figure 4.



- 1 - bacterial proteins synthesis
- 2 - colonization counteraction
- 3 - selective carbon and energy source
- 4 - contention
- 5 - fermentation
- 6 - inhibition
- 7 - butyl acid

Figure 3. Proposed mechanism of prebiotic action

7. Production of prebiotics

Some prebiotics can be extracted from plant sources, but most are synthesized commercially using enzymatic or chemical methods. Overall, prebiotics are manufactured by four major routes (Table 1). Food-grade oligosaccharides are not pure products, but are mixtures containing oligosaccharides of different degrees of polymerization (DP), the parent polysaccharide or disaccharide, and monomer sugars. Oligosaccharide products are sold at this level of purity, often as syrups. Chromatographic purification processes are used to remove contaminating mono- and disaccharides to produce higher purity oligosaccharide products containing between 85 and 99% oligosaccharides, which are often dried to powders [17].

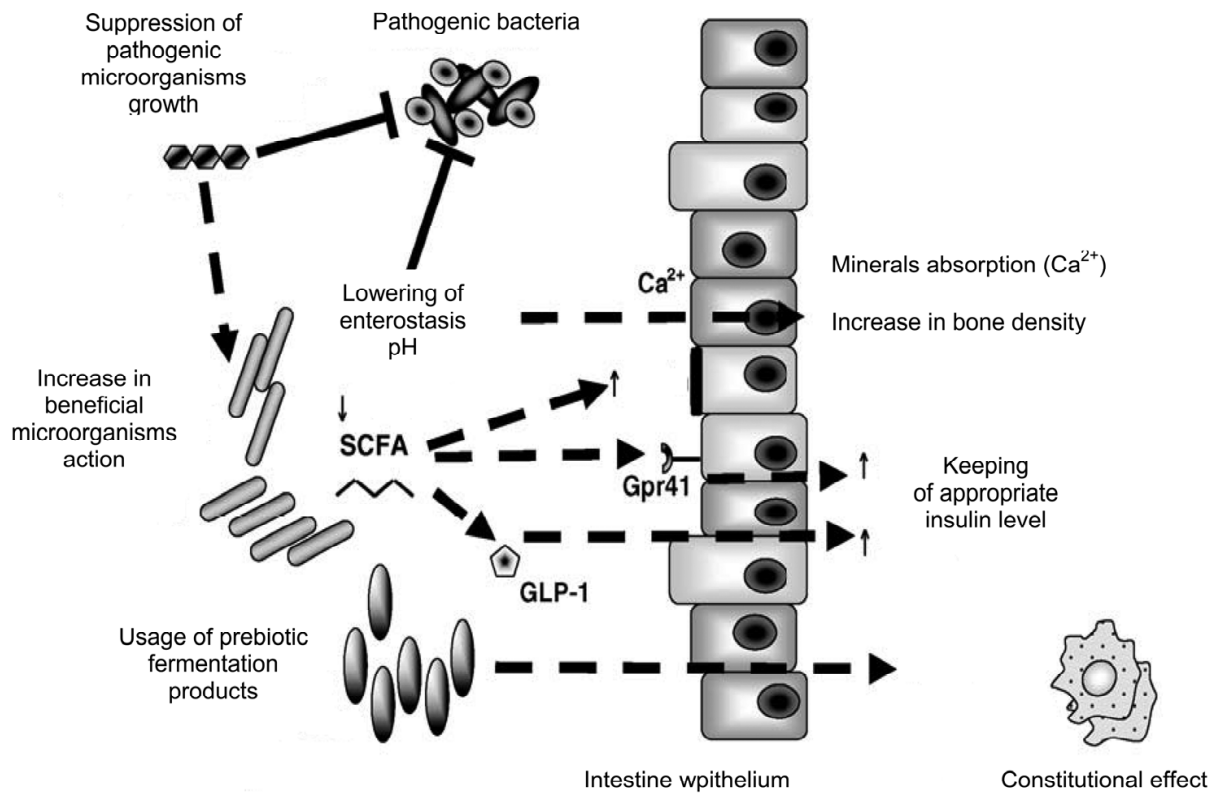


Figure 4. Mechanism of prebiotic action

Approach	Process	Prebiotic Examples
Direct extraction	Extraction from raw plant materials	Resistant starch from maize Inulin from chicory Soybean oligosaccharides from soybean whey
Controlled hydrolysis	Controlled enzymatic hydrolysis of polysaccharides; may be followed chromatography to purify the prebiotics	Fructooligosaccharides from inulin Xylooligosaccharides from arabinoxylan
Transglycosylation	Enzymatic process to build up oligosaccharides from disaccharides; may be followed by chromatography to purify the prebiotics	Fructooligosaccharides from sucrose Galactooligosaccharides from lactose Lactosucrose from lactose + sucrose
Chemical processes	Catalytic conversion of carbohydrates	Lactitol from hydrogenation of lactose Lactulose from alkaline isomerization of lactose

Table 1. Production of prebiotic carbohydrates

Carbohydrate	Chemical structure	Degree of polymerisation	Method of manufacture
Inulin	$\beta(2-1)$ -Fructans	2 - 65	Extraction from chicory root and <i>Agave tequilana</i> .
Fructooligosaccharides (FOS)	$\beta(2-1)$ -Fructans	2 - 9	Transfructosylation from sucrose or hydrolysis of chicory inulin.
Galactooligosaccharides (GOS)	Galactose oligomers and some glucose/ lactose/ galactose units	2 - 5	Produced from lactose by β -galactosidase.
Soya-oligosaccharides	Mixture of raffinose and stachyose	3 - 4	Extracted from soya bean whey.
Xylooligosaccharides (XOS)	$\beta(1-4)$ -Linked xylose	2 - 4	Enzymatic hydrolysis of xylan. Enzyme treatments of native lignocellulosic materials. Hydrolytic degradation of xylan by steam, water or dilute solutions of mineral acids.
Isomaltooligosaccharides (IMO)	$\alpha(1-4)$ -glucose and branched $\alpha(1-6)$ -glucose	2 - 8	Microbial or enzymatic transgalactosylation of maltose. Enzymatic synthesis from sucrose.
Dextrins	Mixture of glucose-containing oligosaccharides	Various	Chemical modification of starch.

Table 2. Main candidates for prebiotic status

Different manufacturing processes also produce slightly different oligosaccharide mixtures. For example, FOS mixtures produced by transfructosylation of sucrose contain oligosaccharides between three and five monomer units, with the proportion of each oligosaccharide decreasing with increasing molecular size. These oligosaccharides contain a terminal glucose with β -1 \rightarrow 2 linked fructose moieties. FOS produced by the controlled hydrolysis of inulin contain a wider range of β -1 \rightarrow 2 fructooligosaccharide sizes (DP 2–9), relatively few of which possess a terminal glucose residue. Even different β -galactosidases used in the production of GOS will produce oligosaccharide mixtures with different proportions of β -1 \rightarrow 4 and β -1 \rightarrow 6 linkages. Hence, there can be some diversity between the structures of oligosaccharides produced by different

manufacturers. The precise impact of these differences in their health effects remains to be determined [17, 100].

There are many oligosaccharides under investigation for their prebiotic potential. Fermentation of some oligosaccharides is not as selective as that of FOS, and their prebiotic status therefore remains in doubt. The main candidates for prebiotic status is provided in Table 2 [26]. There is, therefore, a need for new prebiotic substances of distinct, selective stimulation of growth of lactic acid bacteria, and non-fermented or slightly fermented by other, sometimes pathogenic intestinal bacteria. The search for functional food or functional food ingredients is beyond any doubt one of the leading trends in today's food industry.

8. Resistant dextrins as prebiotics

8.1. Resistant starch

Resistant starch (RS) includes the portion of starch that can resist digestion by human pancreatic amylase in the small intestine and thus, reach the colon. The general behaviour of RS is physiologically similar to that of soluble, fermentable fibre, like guar gum. The most common results include increased faecal bulk and lower colonic pH and improvements in glycaemic control, bowel health, and cardiovascular disease risk factors, so it has shown to behave more like compounds traditionally referred to as dietary fibre [31, 60, 97, 126].

Resistant starch is found in many common foods, including grains, cereals, vegetables (especially potatoes), legumes, seeds, and some nuts [31, 35].

Resistant starch may not be digested for four reasons [30, 38, 60]:

- this compact molecular structure limits the accessibility of digestive enzymes, various amylases, and explains the resistant nature of raw starch granules. The starch may not be physically bio accessible to the digestive enzymes such as in grains, seeds or tubers,
- the starch granules themselves are structured in a way which prevents the digestive enzymes from breaking them down (e.g. raw potatoes, unripe bananas and high-amylose maize starch),
- starch granules are disrupted by heating in an excess of water in a process commonly known as gelatinization, which renders the molecules fully accessible to digestive enzymes. Some sort of hydrated cooking operation is typical in the preparation of starchy foods for consumption, rendering the starch rapidly digestible. However, if these
- starch gels are then cooled, they form starch crystals that are resistant to enzymes digestion. This form of "retrograded" starch is found in small quantities (approximately 5%) in foods such as "corn-flakes" or cooked and cooled potatoes, as used in a potato salad.
- selected starches that have been chemically modified by etherisation, esterisation or cross-bonding, cannot be etherisation, esterisation or cross-bonding, cannot be broken down by digestive enzymes.

Resistance starch is the sum of starch itself and products of her decomposition, that are neither being digested nor absorbed in the small bowel of healthy human [23]. Resistant starch is the difference between amount of the starch exposed to the action of amylolytic enzyme complex and the amount of starch decomposed to glucose during hydrolysis performed by those enzymes [83, 123].

$$RS = TS - (RDS + SDS)$$

Where:

RS – resistant starch

TS – total starch

RDS – rapidly digestible starch

SDS – slowly digestible starch

Few types of digestible starch are recognized nowadays [36, 52, 113].

Resistant starch of type 1 – RS1 covers the starch in plant cells with undestroyed cell walls. This starch is unavailable for digestive enzymes present in human intestinal tract, and thus together with fragments of plant tissues passes through the small bowel getting to the large bowel untouched, and there it can undergo fermentation [70, 104]. RS1 is heat stable in most normal cooking operations, which enables its use as an ingredient in a wide variety of conventional foods [31].

Resistant starch type 2 – RS2 is composed of native starch granules from certain plants containing uncooked starch or starch that was gelatinized poorly and hydrolyzed slowly by R-amylases (e.g., high-AM corn starches). RS2 covers scoops of raw starch of some plant species, especially high-amylase corn, potato and banana [70, 104]. Huge size of raw potato flour scoops and hence combined with it limited area of the access for enzymes was considered as the cause of its resistance [73]. But the main reason for the resistance of the raw starch of some plant species on amylolytic enzymes is the structure of its scoops and crystallization type B that is present within them (in the scoop of potato and corn starch). Also other elements of the scoop structure have an impact on the resistance of the starch – such as the shape of the area, size of pores or susceptibility of the starch to germinate. A particular type of RS2 is unique as it retains its structure and resistance even during the processing and preparation of many foods; this RS2 is called high-AM maize starch [31, 117].

Resistant starch type 3 – RS3 covers the substance precipitated from pap or starch gel during the process of retrograding. During the germination of the starch in the lowered temperature and with the proper concentration (1.5% amylose, 10% amylopectin) colloidal solution is formed. Stable starch phase existing as double helix forms reticular structure binding water phase in its 'eyes'. During the storage of the gel (few hours in lowered temperature) helices undergo aggregation forming thermally stable crystal structures. Such structures show the resistance to amylolytic enzymes [66, 104]. RS3 is of particular interest, because of its thermal stability. This allows it to be stable in most normal cooking operations, and enables its use as an ingredient in a wide variety of conventional foods [15].

During food processing, in most cases in which heat and moisture are involved, RS1 and RS2 can be destroyed, but RS3 can be formed. Storey et al. [99], classified a soluble polysaccharide called 'retrograded resistant maltodextrins' as type 3 RS. They are derived from starch that is processed to purposefully rearrange or hydrolyze starch molecules, and subsequent retrogradation, to render them soluble and resistant to digestion. This process results in the formation of indigestible crystallites that have a molecular similarity to type 3 RS but with a smaller degree of polymerization as well as a lower MW, converting a portion of the normal α -1,4-glucose linkages to random 1,2-, 1,3-, and 1,4- α or β linkages [25, 31,64].

The definition of presented forms of resistant starch may be presented according to the formulas [83]:

$$RS1 = TS - (RDS + SDS) - RS2 - RS3$$

$$RS2 = TS - (RDS + SDS) - RS1 - RS3$$

$$RS3 = TS - (RDS + SDS) - RS2 - RS1$$

Where:

RS1 – resistant starch type 1

RS2 – resistant starch type 2

RS3 – resistant starch type 3

TS – total starch

RDS – rapidly digestible starch

SDS – slowly digestible starch

Resistant starch type 4 – RS4 covers the starch chemically or physically modified and achieved by combination of these two processes. During chemical modification new functional groups are brought into the starch chain, and they bind to glucose residues. Presence of the substituents and spatial changes in the chain prevent proper functioning of human digestive enzymes. In physical method, during warming of starch in high temperature process of dextrinization occurs, and it may also occur in the presence of acid as a catalyst. One of the products of dextrinization is free glucose, which binds to the chains randomly. As a result of such process between glucose residues, bonds typical for starch and those normally not existing in its chains, arise [13, 70, 124].

Resistant starch type 5 – RS5 is an AM-lipid complexed starch [31, 46], which is formed from high AM starches that require higher temperatures for gelatinization and are more susceptible to retrograde [20, 31]. In general, the structure and amount of starch-lipid in foods depend on their botanical sources. Also, Froberg and Quanz [29] defined as RS5 a polysaccharide that consists of water-insoluble linear poly α -1,4-glucan that is not susceptible to degradation by alpha-amylases. They also found that the poly- α -1,4-D-glucans promote the formation of short-chain fatty acids (SCFA), particularly butyrate, in the colon and are thus suitable for use as nutritional supplements for the prevention of colorectal diseases [31].

RS is the fraction of starch which is not hydrolyzed to D-glucose in the small intestine within 120 min of being consumed, but which is fermented in the colon. Many studies have shown that RS is a linear molecule of α -1,4-D-glucan, essentially derived from the retrograded AM fraction, and has a relatively low MW (1.2×10^5 Da) [31].

Resistant starch obtained during chemical or physical modification is being investigated nowadays, due to the fact that it possesses some specific physical properties, as well as because of its health benefits [49, 86, 87, 96]. During chemical modification functional groups are introduced to the starch molecule, which then leads to the changes of physical and chemical properties of obtained product, and also it lowers the availability of the starch to amylolytic enzymes, because new functional groups prevent occurring of the enzyme-substrate complex [7]. Chemical modification was found to be advantageous method of decrease of starch digestion, and therefore starch modified chemically may be the source of resistance starch RS4 [15, 36, 70].

8.2. Resistant dextrin

Resistant dextrins are defined as short chain glucose polymers, without sweet taste and performing strong resistance to hydrolytic action of human digestive enzymes [68]. In accessible throughout the whole products (resistant dextrin Nutriose, Fibersol) bigger percentage presence of (1 \rightarrow 2)-, (1 \rightarrow 3)-, (1 \rightarrow 6)- α and β -glycoside bonds than in native starch which is the source of getting them [62, 115].

During warming of starch in high temperature, with or without addition of catalyst (usually acidic) dextrinization of starch is observed. Dextrinization is a complex process taking chemical side of it into account. It covers depolymerization, transglucolization and repolymerization [122].

During warming of wet starch random bonds (1 \rightarrow 4) and rarely (1 \rightarrow 6) hydrolytically break. Intermediate form in this reaction is either oxycarbonic ion, or free radicals [105].

Most probably, dextrinization undergoes the mixed mechanism. In case of warming of dry starch (or with a low moisture content), bonds (1 \rightarrow 6) are made between two starch chains and intramolecular dehydration coincides, which results in development of 1,6-anhydro- β -D-glucose. In such a way only extreme glucose units with free hydroxyl group within anomeric carbon atom may react. In both cases exuded water has hydrolytic character. With the temperature equal to about 290°C α -(1 \rightarrow 4)-glycosidic bonds begin to break. However during dextrinization not only bond breaking is observed, but also the isomerization (e.g. through mutarotaion) or formation of new bonds. Hydroxyl groups at C-2, C-3 or C-6 glucose unit act on oxycarbonic ions or free radicals and transglycolization, which is based on formation of (1 \rightarrow 2), (1 \rightarrow 3) and (1 \rightarrow 6) bonds, undergoes. This process leads to formation of branched dextrins. Because of spherical considerations, and maybe also thermodynamic ones, formation of (1 \rightarrow 6) bonds is privileged. 1,6-anhydro- β -D-glucose is formed, which easily forms polymers, leads to formation of 4-O- α -D-, 4-O- β -D-, 2-O- α -D- and 2-O- β -D-glukopiranozylo-1,6-dihydro- β -D-glucopyranose [105, 122].

In the next stage following reactions have to be taken into account:

- reversion, that is the reaction between glucose units leading to formation of (1→6)-glycosidic bonds,
- reaction between (1→6)-anhydro-β-D-glucopyranose and free radicals with formation of 1,6-glycosidic bonds,
- recombination.

From all presented reactions the most characteristic and dominating one is transglycolisation. Formation of bonds other than typical for starch (1→4) and (1→6) causes that the received product becomes unavailable for human digestive enzymes and shows properties of resistant starch.

In the presence of acidic catalyst dextrinization process progresses a bit differently. First the hydrolysis undergoes, and in the result of it (1→6) bonds break, but (1→4) bonds stay untouched. In this way, white dextrins are formed. Because (1→6) bonds are more resistant to hydrolysis than (1→4) bonds, these last ones undergo transformation into (1→6) bonds [105].

The role of basic catalyst in dextrinization process is not well known. The only thing know is that in this process deprotonating of hydroxyl groups at C-2 and C-3 is the first stage. In the presence of oxidating agents atom C-1 of terminal glucose units are being oxidized to carboxyl group [105]. Summing up, as a result of hydrolysis the reductive ends of the starch become glucose cations, which undergo intramolecular dehydration forming (1→6)-anhydro-β-D-glucopyranose unit or they take part in formation of intermolecular bonds (transglycolization). As a result of this process random glycosidic (1→2), (1→3) bonds are formed [68]. Formation of bonds other than typical for starch, i.e. (1→4) and (1→6) causes that end product doesn't undergo hydrolysis through the human digestive enzymes [115].

In the process of formation of resistant dextrin, piroconversion is the first stage, and it covers following steps: thermolysis, transglucolysis, regrouping and repolymerisation. Starch thermolysis leads to breaking of α-D-(1→4) and α-D-(1→6) glycosidic bonds, which then leads to the formation of products with lower molecular mass and higher viscosity and reducing sugars content. After transglucolysis recombination of hydrolyzed starch fragments with free hydroxyl groups happens, and formation of strongly branched structures. Repolymerisation of glucose and oligosaccharides with formation of high molecular compounds is done in high temperature and presence of acidic catalyst (e.g. hydrochloric acid) [68]. Achieved pirodextrins are the mixture of poli- and oligosaccharides with a different degree of polymerisation (DP), and simultaneously with different molecular mass. Pirodextrins are subjected to enzymatic hydrolysis or chromatography – stages, which aim is to reduce the fractions other than typical for the starch (i.e. containing bonds other than α-D-(1→4) and α-D-(1→6) glycosidic ones [6, 81, 118].

Chemical modification has long been known to inhibit in vitro digestibility of starch, the extent of which is related to the type and degree of modification, the extent of gelatinization, and the choice of enzyme [117, 119]. Starch phosphates [45], hydroxypropyl starches [51,

121], starch acetates [120], phosphorylated starch [86, 94, 119], and citrate starches [117, 124] have been tested for enzymatic degradation previously. In previous studies have been suggested that the substituted groups hindered enzymatic attack and thus also made neighboring bonds resistant to degradation. Chemical substitution of starch reduces its enzyme digestibility, probably because the bulky derivatizing groups sterically hinder formation of the enzyme-substrate complex [7]. The largest change in digestibility has been achieved through cross-linking of starch [119]. The application of organic acids, as citric acid and tartaric acid as derivatizing agents seemed to be profoundly safe. These acids are nutritionally harmless compared to other substances used for chemical modification [123]. When citrate starches were fed to rats no pathological changes could be found in comparison to native wheat and corn starches [117].

Potato starch in a natural form has limited possibilities to be used, and its chemical structure and physical properties give possibilities to many modifications, also those leading to formation of resistant substances to amylolytic enzymes. Big hopes are laid on usage of products with modified starch, especially resistant starch and resistant dextrin as substances with prebiotic properties.

Kapusniak et al. [47] resistant dextrin was receive by simultaneous pyroconversion and chemical modification (esterification/ cross-linking) of potato starch in the presence of hydrochloric acid as catalyst of dextrinization process, and citric acid as derivatizing agent. Potato starch was modified by thermolysis in the presence of acid catalyst in a sealed container at 130°C for 180 min. The effect of addition of multifunctional polycarboxylic acids (citric and tartaric) on the progress of dextrinization process, structure and properties of resulting products was investigated [47]. It seems likely that probiotic activity will be exhibited by dextrin obtained by simultaneous thermolysis and chemical modification of potato starch in the presence of a volatile inorganic acid (hydrochloric acid) as a catalyst of the dextrinization process and an excess amount of an organic acid (tartaric acid) as a modifying factor. Kapuśniak et al. [47, 48] analyzed this dextrin in terms of the solubility and pH of its 1% aqueous solution, the content of reducing sugars, molecular mass distribution, weight average molecular mass using high performance size-exclusion chromatography (HPSEC), average chain length using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), and the content of the resistant fraction using the enzymatic-gravimetric method AOAC 991.43, the enzymatic-gravimetric-chromatographic method AOAC 2001.03 [69], the enzymatic-spectrophotometric method [23] and the pancreatin-gravimetric method [94]. It was shown that the use of tartaric acid in the process of starch thermolysis yielded acidic dextrin characterized by high water solubility (about 68%) and a high content of reducing sugars (about 29%). The studies showed that dextrin modified with tartaric acid did not contain any traces of unreacted starch, and the percentage share of the main fraction (having a weight average molecular mass of about 1.800 g/mol) was 80%. The average length of the carbohydrate chain in dextrin obtained with tartaric acid was 8.2 as determined by means of HPAEC. A study by Kapuśniak et al. [47] revealed that the content of the resistant fraction in dextrin modified with tartaric acid, determined by means of the AOAC 991.43, amounted

to 44.5%. However, results obtained by the Engllyst [43] enzymatic-spectrophotometric method showed that the actual content of the resistant fraction was above 68%. Kapuśniak et al. [47, 48] used the official AOAC 2001.03 method to determine the content of the resistant fraction in dextrin modified with tartaric acid. This method is the latest approved method for determining total content of dietary fiber in foods containing resistant maltodextrins. Apart from measuring the content of insoluble dietary fiber and the high molecular weight fractions of soluble fiber, this method makes it possible to determine resistant oligosaccharides (by using high-performance liquid chromatography, HPLC). The total content of dietary fiber in dextrin modified with tartaric acid was about 50% [47, 48]. In the Engllyst method, fractions undigested after 120 min are considered resistant. In the pancreatin-gravimetric method, similarly as in the Engllyst method, samples are digested with pancreatin, but resistant fractions are determined gravimetrically only after 16 h. In the case of dextrin modified with tartaric acid, the results of determination by the pancreatin-gravimetric method (67%) were similar to those obtained in the previous studies using the Engllyst method (68%), but much higher than those obtained using the AOAC 2001.03 method (50%) [43, 47]. The observed differences among the various methods in terms of the measured content of the resistant fraction in dextrin modified with tartaric acid was caused by the fact that, according to the latest reports, enzymatic-gravimetric methods (including AOAC 2001.03) using thermostable α -amylase can determine only part of resistant starch type 4 [124]. Based on the enzymatic tests, it can be argued that dextrin obtained using an excessive amount of tartaric acid may be classified as resistant starch type 4.

8.3. Prebiotic effects of resistant starch and resistant dextrin

Resistant starch has a long history of safe consumption by humans and is a natural component of some foods. Intakes vary but are generally low, particularly in Western diets. Similar to soluble fibre, a minimum intake of resistant starch (5 - 6 g) appears to be needed in order for beneficial reductions in insulin response to be observed. Estimates of daily intake of resistant starch range from 3 to 6 g/day (averaging 4.1 g/day) [4, 31]. As a food ingredient, resistant starch has a lower calorific (8 kJ/g) value compared with fully digestible starch (15 kJ/g) [31, 78], therefore it can be a substitutive of digestible carbohydrates, lowering the energy content of the final formulation. Some resistant starch products are also measured as total dietary fibre in standard assays, potentially allowing high-fibre claims [31, 35].

Resistant starch can be fermented by human gut microbiota, providing a source of carbon and energy for the 400 - 500 bacteria species present in this anaerobic environment and thus potentially altering the composition of the microbiota and its metabolic activities. The fermentation of carbohydrates by anaerobic bacteria yields SCFA, primarily composed of acetic, propionic, and butyric acids, which can lower the lumen pH, creating an environment less prone to the formation of cancerous tumours [31, 126].

RS consumption has also been related to reduced post-prandial glycemic and insulinemic responses, which may have beneficial implications in the management of diabetes, and is

associated with a decrease in the levels of cholesterol and triglycerides. Other effects of resistant starch consumption are increased excretion frequency and faecal bulk, prevention of constipation and hemorrhoids, decreased production of toxic and mutagenic compounds, lower colonic pH, and ammonia levels. Considering that nowadays several diseases result from inadequate feeding, and that some may be related to insufficient fibre intake, it is reasonable to assume that an increased consumption of indigestible components would be important [31].

Resistant starch enhance the ileal absorption of a number of minerals in rats and humans. Lopez et al. [59] and Younes et al. [128] reported an increased absorption of calcium, magnesium, zinc, iron and copper in rats fed RS-rich diets. In humans, these effects appear to be limited to calcium [16, 30, 107]. Resistant starch could have a positive effect on intestinal calcium and iron absorption. A study to compare the apparent intestinal absorption of calcium, phosphorus, iron, and zinc in the presence of either resistant or digestible starch showed that a meal containing 16.4% RS resulted in a greater apparent absorption of calcium and iron compared with completely digestible starch [30, 65].

Liu and Xu [58] showed that resistant starch dose-dependently suppressed the formation of colonic aberrant crypt foci only when it was present during the promotion phase to a genotoxic carcinogen in the middle and distal colon, suggesting that administration of resistant starch may retard growth and/or the development of neoplastic lesions in the colon. Therefore, colon tumorigenesis may be highly sensitive to dietary intervention. Adults with preneoplastic lesions in their colon may therefore benefit from dietary resistant starch. This suggests the usefulness of resistant starch as a preventive agent for individuals at high risk for colon cancer development [30, 58].

Short chain fructooligosaccharides (FOS) and resistant starch (RS) may act synergistically (by combining, and thus increasing, their prebiotic effects) [31, 79], the administration of the combination of FOS and RS induced changes in the intestinal microbiota, by increasing lactobacilli and bifidobacteria in caecum and colonic contents. Several types of prebiotic fibres can be distinguished considering their rate of fermentability. Such role depends on the carbohydrate chain length as it has been demonstrated *in vitro* in a fermentation system, showing that FOS are rapidly fermented whereas long chain prebiotic, like inulin, are steadily fermented. These observations have been confirmed *in vivo* once the different prebiotics reach the large intestine: FOS are rapidly fermented, whereas RS is slowly degraded. In consequence, the particular kinetics would determine the region of the intestine where the effects will be clearer. Thus, FOS would be more active in the first parts of the large bowel whereas RS would reach the distal part of the colon. In fact, Le Blay et al. [31, 50] have reported that administration of FOS or raw potato starch induces different changes in bacterial populations and metabolites in the caecum, proximal, and distal colon, as well as in faeces. As compared with RS FOS doubled the pool of faecal fermentation products, like lactate, while the situation was just the opposite distally. These observations confirm that each prebiotic shows particular properties, which should be considered before their application for intestinal diseases; thus, rapidly fermentable prebiotics are particularly

useful in those affecting the proximal part of the large intestine, while slowly fermentable prebiotics should be chosen for more distal intestinal conditions. Moreover, an association with different prebiotics with complementary kinetics should be considered when a health-promoting effect throughout the entire colon is required. So, functional foods based on the combination of two different dietary fibres, with different rate of fermentability along the large intestine, may result in a synergistic effect, and thus, in a more evident prebiotic effect that may confer a greater health benefit to the host [31, 127].

Younes et al. [127] was to examine the potential synergistic effect of a combination of these two fermentable carbohydrates (inulin and resistant starch). For this purpose, thirty-two adult male Wistar rats weighing 200 g were used in the present study. The rats were distributed into four groups, and fed for 21 d a fibre-free basal purified diet or diet containing 100 g inulin, or 150 g resistant starch (raw potato starch)/kg diet or a blend of 50 g inulin and 75 g resistant starch/kg diet. After an adaptation period of 14 d, the rats were then transferred to metabolic cages and dietary intake, faeces and urine were monitored for 5 d. The animals were then anaesthetized and faecal Ca and Mg absorption were measured. Finally, the rats were killed and blood, caecum and tissues were sampled. Ca and Mg levels were assessed in diets, faeces, urine, caecum and plasma by atomic absorption spectrometry. The inulin and resistant starch ingestion led to considerable faecal fermentation in the three experimental groups compared with the control group diet. Moreover, both carbohydrates significantly increased the intestinal absorption and balance of Ca and Mg, without altering the plasma level of these two minerals. Interestingly, the combination of the studied carbohydrates increased significantly the faecal soluble Ca and Mg concentrations, the apparent intestinal absorption and balance of Ca, and non-significantly the plasma Mg level. The combination of different carbohydrates showed synergistic effects on intestinal Ca absorption and balance in rats [127].

The example of commercially available resistant dextrin is Nutriose. It is a non-viscous soluble fiber made from starch using a highly controlled process of dextrinization. It is mostly resistant to digestion in the small intestine and largely fermented in the colon. A process of dextrinization includes a degree of hydrolysis followed by repolymerization that converts the starch into fiber by forming non digestible glycosidic bonds. Nutriose is totally soluble in cold water without inducing viscosity [37, 53]. It is produced from wheat or maize starch using a highly controlled process of dextrinization followed by chromatographic fractionation step [28]. Nutriose FB®06, produced from wheat starch, contains approx. 13% of 1,2- and 14% of 1,3- glycosidic linkages [81]. The weight average molecular weight (M_w) and the number average molecular weight (M_n) for that dextrin were nearly 5000 and 2800 g/mole respectively. The residual content of sugars (DP1-2) of Nutriose FB®06 was below 0.5% and it could be considered as sugar free [80]. The enzyme-resistant fraction content, determined according to AOAC official method 2001.03 for total dietary fiber in foods containing resistant maltodextrins, was nearly 85% for Nutriose®06 and nearly 70% for Nutriose®10 [80, 81]. Nutriose has found wide application in food and pharmaceutical industries, as components of fiber-enriched drinks [92], components of a fiber-enriched composition for enteral nutrition [88], granulation binders [93], in the preparation of low-

calorie food [11], and sugar-free confectionery [91]. Very well tolerated, Nutriose may be 20–25% of a product's composition without causing discomfort or bloating. About 15% of Nutriose are absorbed in the small intestine, about 75% fermented in the large intestine, while the remainder (about 10%) is excreted in the faeces [72, 110]. Nutriose induced an increase of the colonic saccharolytic flora and decrease in potentially harmful *Clostridium perfringens* in human faeces [54, 72]. Nutriose induced a decrease in the faecal pH of human volunteers, increased production of short chain fatty acids (SCFAs) in rats [54], induced changes in faecal bacterial enzyme concentration [72, 110]. It was also shown that learning (respectively physical) performances are improved in rats 180 minutes (respectively 150 minutes) after the consumption of Nutriose, compared to dextrose. The glycaemic kinetics is not sufficient to predict this effect: even though the glycaemic peak was lower with the resistant dextrin than with dextrose, the glycaemia was the same between the two groups at 150 and 180 minutes after ingestion. These preliminary results are very encouraging [82].

Guérin-Deremaux et al. [37] found that the non-viscous soluble dietary fiber may influence satiety. The randomized, double-blind, placebo-controlled clinical study in 100 overweight healthy adults in China investigated the effect of different dosages of dietary supplementation with a dextrin, Nutriose, on short-term satiety over time. Subjects were randomized by body mass index and energy intake and then assigned to receive either placebo or 8, 14, 18, or 24 g/d of Nutriose mixed with orange juice (n = 20 volunteers per group). On days - 2, 0, 2, 5, 7, 14, and 21, short-term satiety was evaluated with a visual analog scale, and hunger feeling status was assessed with Likert scale. Nutriose exhibits a progressive and significant impact on short-term satiety, which is time and dosage correlated. Some statistical differences appear for the group 8 g/d from day 5, and from day 0 for the groups 14, 18, and 24 g/d. The hunger feeling status decreases significantly from day 5 to the end of the evaluation for the group 24 g and from day 7 for the groups 14 and 18 g. By day 5, the group 24 g showed significantly longer time to hunger between meals compared with placebo. These results suggest that dietary supplementation with a soluble fiber can decrease hunger feeling and increase short-term satiety over time when added to a beverage from 8 to 24 g/d with time- and dose-responses relationship [37].

Human clinical trials on healthy subjects have shown that Nutriose is well tolerated and able to stimulate the growth of acid-resistant bacteria. We particularly observed a beneficial shift in the bacterial microbiota profile to butyrogenic genera such as *Peptostreptococcus*, *Fusobacterium* and *Bifidobacterium* [37].

Resistant maltodextrins made from starch are commercially available. Fibersol-2 is well-known soluble, non-digestible, starch-derived resistant maltodextrin [67]. Fibersol-2 is produced from corn-starch by pyrolysis and subsequent enzymatic treatment (similar to the process to manufacture conventional maltodextrins) to convert a portion of the normal α -1,4 glucose linkages to random 1,2-, 1,3- α or β linkages [69]. Solubility of Fibersol-2 in water reaches 70% (w/w) at 20°C. It is readily dispersible in water and highly compatible with dry drink mix applications. At typical use levels, it yields clear, transparent solutions that are near water-like in performance. Fibersol-2 adds no flavor or odor. It has essentially no

sweetness of its own. It shows stability to acid and heat/retort processing, including stability in high acid, hot filled, aseptic, or retorted products like juices, sauces, puddings, fluid milks, and sports. Fibersol-2 shows superior freeze-thaw stability. It shows precise and extremely low viscosity and very low hygroscopicity. It does not actively participate in non-enzymatic Maillard-type browning [69]. Fibersol-2 exhibited very important physiological properties. It was fermented slowly, producing less acid and gas than most soluble dietary fiber [27]. Studies indicated that Fibersol-2 could effectively reduce postprandial levels of blood glucose and insulin [109]. Fibersol-2 significantly reduced levels of blood triglycerides and serum cholesterol [125]. By adding stool volume, moisture, and reducing transit time, Fibersol-2 helped maintain good colon health, potentially reducing the incidence of various types of colon diseases and cancers [102]. Fibersol-2 effectively promoted the growth of a variety of beneficial bacteria (naturally occurring or ingested as probiotics) in the colon. In promoting the growth of beneficial bacteria, Fibersol-2 indirectly reduced the presence of undesirable bacterial species [41, 63].

Bodinhah et al. [8] found that a non-viscous resistant starch significantly lowered energy intake after intake of the supplement compared with placebo during both an ad libitum test meal ($P = 0.033$) and over 24 hours ($P = 0.044$). Cani et al [14] found that treatment with the fermentable dietary fiber oligofructose increased satiety after breakfast and dinner and reduced hunger and prospective food consumption after dinner, suggesting a role for the use oligofructose supplements in the management of food intake in overweight and obese patients [37].

Kapusniak et al. [47] and Śliżewska et al. [98] enzyme-resistant chemically modified dextrins resulting from heating of potato starch with hydrochloric acid as catalyst and additionally polycarboxylic acids (citric and tartaric acids) were tested as the source of carbon for probiotic bacteria (*Lactobacillus* and *Bifidobacterium*) and bacteria isolated from the human feces (*Escherichia coli*, *Enterococcus*, *Clostridium* and *Bacteroides*). It was shown that all of the tested bacteria, both probiotics and those isolated from human feces, were able to grow and utilize dextrin as a source of carbon, albeit to varying degrees. After 24 h the highest growth was recorded for the probiotic bacteria *Lactobacillus* and *Bifidobacterium*, the weakest for *Clostridium* and *Escherichia coli* bacteria. After prolonging culture time to 72–168 h, which corresponds to retarded or pathological passage of large intestine contents, the viability of intestinal bacteria in a medium with resistant dextrin was found to be lower by one or two orders of magnitude as compared to the viability of probiotic bacteria. The number of probiotics and bacteria isolated from fecal samples grown in media containing 1% glucose was lower by two or three orders of magnitude than that of corresponding bacteria grown in a medium containing dextrin. This may have been caused by lower pH values of the controls, in which the culture environment became unfavorable to preserving high viability by the studied bacteria. This may have also been caused by the protective effects of dextrin on the bacteria. After the completion of incubation, that is, at 168 h, lactobacilli and bifidobacteria were found to be highly viable. Their counts were higher by one or two orders of magnitude than those of the intestinal bacteria *E. coli*, *Enterococcus*, *Clostridium* and *Bacteroides* isolated from fecal samples. At 168 h of incubation, the probiotic bacteria

amounted to over 44% of the whole population. The *Clostridium* strain showed the weakest growth, with a 9.5% share in the entire population, while *Enterococcus*, *Escherichia coli*, and *Bacteroides* amounted to from 15.3% to 15.8% of the population [48].

9. Conclusions

In today's world, life style is an important determinant of health in later life. Lack of physical activity, particularly if associated with over consumption, increases the risk of development of nutrition related chronic diseases, such as obesity, hypertension, cardiovascular diseases, osteoporosis, type II diabetes, and several cancers. Over the last decade, drastic changes have taken place in the image and assessment of the importance of the daily diet. Foods are no longer judged in terms of taste and immediate nutritional needs, but also in terms of their ability to improve the health and well-being of consumers. The role of diet in human health has led to the recent development of the so-called functional food concept. A functional food is dietary ingredient, that has cellular or physiological effects above the normal nutritional value. Functional food can contain probiotics and/or prebiotics [33, 40].

The studies suggests that soluble fibers help to regulate the digestive system, may increase micronutrient absorption, stabilize blood glucose and lower serum lipids, may prevent several gastrointestinal disorders, and have an accepted role in the prevention of cardiovascular disease. It is concluded that supplementation with soluble fibers (e.g. wheat dextrin) may be useful in individuals at risk of a lower than recommended dietary fiber intake.

A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or, a limited number, of bacteria in the colon that can improve host health [19]. Some carbohydrates, such as fructooligosaccharides (FOS) [10, 34, 75, 101], inulin [75, 76, 77, 112] and galactooligosaccharides (GOS) [84, 108] are well-accepted prebiotics.

Promising sources of prebiotics are starch products, especially resistant starch (RS) [3, 19, 106] and products of partial degradation of starch [56].

The commercial degraded starches are known as converted starches and comprise the "thin-boiling" acid-converted starches, oxidized starches, and dextrins. There are four major groups of dextrins: maltodextrins produced by hydrolysis of dispersed starch by action of liquifying enzymes such as amylase, degradation products by acid hydrolysis of dispersed starch, cyclodextrins, and pyrodextrins produced by the action of heat alone or in a combination with acid on dry granular starch. On the market pyrodextrins are available in three major varieties: British gums, white dextrins and yellow dextrins [5, 105, 122].

In particular, almost every food oligosaccharide and polysaccharide has been claimed to have prebiotic activity, but not all dietary carbohydrates are prebiotics. Conventional fibers, like pectins, cellulose, etc. are not selectively metabolized by gut bacteria. Resistant

maltodextrins, being a mixture of fractions of different molecular weight (different degree of polymerization), are dietary fibre, but there are not necessarily selective for desirable bacteria in the gut. Hence, research showing the effect of prebiotic should continue to be performed, both in the *in vitro* and *in vivo*.

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