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Resistant starch and “the butyrate revolution”

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Early epidemiological studies indicated that populations that consume a high proportion of non-starch polysaccharide (NSP) dietary fibre (DF) in their daily diet suffer less from gastrointestinal diseases, in particular colorectal cancers, than populations that consume diets that are high in fat and protein but low in NSP fibre. In this respect, diet, by increasing the amount of vegetables and NSP DF’s, has been suggested to contribute as much as 25–35% to risk reduction for colorectal cancer. A reduction of fat intake may further reduce the risk by 15–25%. Based on these observations, DF’s and substances that are part of the fibre complex such as antioxidants, flavonoids, sulphur containing compounds and folate have been proposed as potentially protective agents against colon cancer. However, results from controlled prospective studies in which beta-carotene and vitamin E or isolated dietary fibres were given to high risk groups showed disappointing results. There are recent indications that the regular consumption of certain subclasses of highly fermentable dietary fibre sources result in gut associated immune and flora modulation as well as a significant production of short chain fatty acids. In vitro studies as well as animal studies indicate that in particular propionate and butyrate have the potential to support the maintenance of a healthy gut and to reduce risk factors that are involved in the development of gut inflammation as well as colorectal cancer. A suggestion put forward is that beneficial effects may be obtained in particular by the consumption of resistant starch (RS) because of the high yield of butyrate and propionate when fermented. These SCFA are the prime substrates for the energy metabolism in the colonocyte and they act as growth factors to the healthy epithelium. In normal cells butyrate has been shown to induce proliferation at the crypt base, enhancing a healthy tissue turnover and maintenance. In inflamed mucosa butyrate stimulates the regeneration of the diseased lining of the gut. In neoplastic cells butyrate inhibits proliferation at the crypt surface, the site of potential tumour development. Moreover, models of experimental carcinogenesis in animals have shown the potential to modify a number of metabolic actions and steps in the cell cycle in a way that early events in the cascade of cancer development may be counteracted while stages of progression may be slowed down. The present review highlights a number of these aspects and describes the metabolic and functional properties of RS and butyrate.

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adhere to the intestinal wall of the small intestine and to colonise the large intestine, are examples of such foods.

Prebiotics are another example. Prebiotics are defined as “non-digestible food substances that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacterial species already resident in the colon and thus attempt to improve the host health (Gibson & Roberfroid, 1995). Non-digestible carbohydrates/dietary fibres, such as specific oligosaccharides, are examples of prebiotics.

One of the factors that is being recognised to be of major importance for the maintenance of a healthy digestive system is the colonic flora, especially its bacterial composition and the “nutrients” that it metabolises.

This micro-flora is important for the production and absorption of a number of essential nutrients and for the competition with harmful bacteria, to reduce their negative impact on health. Through the consumption of fermentable carbohydrates the flora may also create a metabolic environment that supports the elimination of toxic and carcinogenic substances. Indeed, a substantial number of studies have pointed to the fact that a desired modification of the intestinal flora and physiology is possible (Gibson & Roberfroid, 1995). Although hard clinical endpoints on beneficial long-term effects are difficult to measure, it is generally believed that pre- and prebiotics may contribute to overall health maintenance (Conway, 2001).

The dietary fibre challenge

A few decades ago it was observed in epidemiological studies that populations that consume a high proportion of non-starch polysaccharide (NSP) dietary fibre (DF) in their daily diet suffer less from gastrointestinal diseases, in particular colorectal cancers, than populations that consume diets that are high in fat and protein but low in NSP fibre. This has lead to thousands of publications on the significance of an increased DF consumption for intestinal health and the prevention of colon cancer.

Although DF was classically defined as non-starch polysaccharides that are not digested in the small intestine and enter the colon to be fermented, it is now recognised that many other sources of non-digestible carbohydrates fall under this definition as well. Examples are different types of non-digestible oligosaccharides and resistant starch (Gibson, Willems, Reading, & Collins, 1996; Champ et al., 2002), even though these are not measured by the current AOAC method for the determination of DF (Champ, Langkilde, Brouns, Kettitz, & Le Bail-Collet, 2002).

Epidemiological studies have shown that environmental factors are the main reasons for geographical differences in colorectal cancer incidence. It was observed that populations that consume diets high in meat and fat have a higher cancer incidence than populations that consume diets high in starch and low in fat and meat (Bingham, 1996). It was also shown that the low incidence populations had mainly cancers in the upper part of the colon whereas high incidence populations had mainly colorectal cancers in the lower part of the large bowel. This leads to the suggestion that the majority of large bowel cancers can potentially be prevented by modification of environmental factors. In this respect, diet has been suggested to contribute as much as 25–35% to risk reduction for colorectal cancer by increasing the amount of vegetables and NSP DF’s. A reduction of fat intake may further reduce the risk by 15–25% (Bingham, 1996). Based on these observations, DF’s and substances that are part of the fibre complex such as antioxidants, flavonoids, sulphur containing compounds and folate have been proposed as potentially protective agents against colon cancer.

However, results from controlled prospective studies, in which beta-carotene and vitamin E or isolated dietary fibres were given to familial multiple polyposis cohorts or adenomatous polyps patients, showed disappointing results (Bingham, 1996; Fuchs et al., 1999; Trock et al., 1990). Accordingly, more recent prevention programmes have been focussed primarily on aspects of a healthy general lifestyle (Wasan & Goodlad, 1996).

The disappointing results mentioned above may be subject to several explanations such as:

- the different types of DF used,
- the absence of other dietary components that normally are part of the DF complex but get lost with the isolation of DF,
- the “basal diet”,
- the chosen biomarkers and
- the stage of colon adenoma development/carcinogenesis.

However, there are indications that certain subclasses of highly fermentable dietary fibre, such as specific oligosaccharides and resistant starch, that result in significant flora modulation and production of short chain fatty acids, in particular propionate and butyrate, may have the potential to reduce risks of developing colorectal cancer.

In this respect, it is interesting to note that Cassidy, Bingham, and Cummings (1994) found a significant negative correlation between the consumption of starch and with it (assuming an RS fraction of 5% within total starch consumed) resistant starch on colorectal cancer incidence. In her study she assessed the starch consumption of populations in 12 countries as measured in individual surveys. A positive correlation with fat and protein consumption was observed but only a weak and non-significant correlation with NSP intake. There was, however, a strong inverse correlation between colorectal cancer incidence and starch intake ($r = -0.70$) and this association was maintained after controlling for partial correlation due to meat and fat consumption.
A suggestion put forward is that this beneficial effect may be related to the fact that the RS consumed is subject to complete fermentation, particularly leading to high levels of butyrate (molar quantity in SCFA produced, 20–28%) compared to slow and less fermentable fibers such as NSP (10–15%) in the large bowel.

Health aspects of resistant starch consumption
This has lead to an immediate growing interest in the field of gut health related to the consumption of Resistant Starch (RS) (Asp, van Amelsvoort, & Hautvast, 1998; Silvester, Bingham, Pollock, 1998; Cumming et al., 1996; Hylla, Gastner, Dusel, Record, 1996; Bird, Brown, & Topping, 2000; Conway, siology, flora and fermentation (Baghurst, Baghurst, & Bird, 1999). Recently a number of publications have highlighted the physiological properties as well as the effects of resistant starches on intestinal digestion, propionate and butyrate, rather than the presence of dietary fibre itself determine the physiology in the large intestine and the resulting health effects (Archer, Meng, Shei, & Hodin, 1998; Goodlad, Ratcliffe, & Fordham, 1989; Scheppach, Gamet, Daviaud, Denis-Pouxviel, Remesy, & Murat, 1992; Goodlad, Ratcliffe, & Fordham, 1989; Scheppach, 1994, 1995; Smith, Yokoyama, & German, 1998; Thornton, Dryden, Kelleher, & Losowsky, 1987).

Scientific studies support the view that retrograded resistant starch (RS) is the most powerful butyrate producing substrate (Bird, Brown, & Topping, 2000; Topping & Bird, 1999). Recently a number of publications have highlighted the physiological properties as well as the effects of resistant starches on intestinal digestion, physiology, flora and fermentation (Baghurst, Baghurst, & Record, 1996; Bird, Brown, & Topping, 2000; Conway, 2001; Cummings et al., 1996; Hylla, Gastner, Dusel et al., 1998; Silvester, Bingham, Pollock et al., 1995; Topping & Bird, 1999). For a summary of the potential actions of RS as derived from in vitro, animal and human studies described in the reviews that are listed above, see Table 1.

Resistant starch consumption
Originally* resistant starch is classified as 3 types (Euresta):

1. RS Type I: starch that is physically inaccessible for digestive enzymes because it is “packed” in fiber material, e.g. grains and seeds.
2. RS Type II: raw starch granules as present in raw potato and green banana.
3. RS Type III: starch mainly represented as recrystallized (retrograded) amylase. This type is mainly present in cooked and subsequently cooled potato, in bread and in corn flakes.

The resistance of these 3 RS types is attributed to particular physical structures, whilst molecular parameters of the starch (α-glucose units connected by α-1,4/α-1,6 glucosidic bonds) remain substantially unchanged. *Recently also some papers mention a category IV covering some chemically modified starches. In this case the resistance to digestion is due to chemical changes in the starch back bone.

There are some indications that RS consumption has historically declined. A French study showed a reduction from initially 7–9 to 3–7 g/day over a period of 40 years, most probably because of changed food habits related to a rapidly industrialised world and a reduced bread consumption (Fig. 1, Brousseau, Dufour, & Volatier, 1998). In comparison, mean current intakes in Western countries (Europe as well as Australia) range from about 4 to 10 g/day, which is comparable to intakes in Australia (Brousseau et al., 1998; Brighent, Casiraghi, & Baggio, 1998, Baghurst et al., 1996). However, some individuals, who consume high amounts of starch containing foods, may have a daily intake as high as 30–40 g/day. Whilst no specific recommendations exist for RS intake, some experimental studies suggest that intakes in the order of ≥ 20 g/day RS may be needed to obtain some of the bowel related benefits (Baghurst et al., 1996).

### Table 1. Functional potential of resistant starches

<table>
<thead>
<tr>
<th>Effects on intestinal flora and metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Completely fermented by intestinal flora</td>
</tr>
<tr>
<td>• Low levels of gas formation when being fermented</td>
</tr>
<tr>
<td>• Elevates colonic butyrate levels more than NSP's when being fermented</td>
</tr>
<tr>
<td>• Reduces intestinal pH in dose dependent manners</td>
</tr>
<tr>
<td>• Selectively utilised by lactobacilli and bifidobacteria</td>
</tr>
<tr>
<td>• Promotes colonisation of lactobacilli and bifidobacteria</td>
</tr>
<tr>
<td>• Reduces intestinal pathogen levels</td>
</tr>
<tr>
<td>• Reduces secondary bile acids</td>
</tr>
<tr>
<td>• Reduces faecal water toxicity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effects on health, gut function and physiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Reduces symptoms of diarrhoea (duration and fluid loss)</td>
</tr>
<tr>
<td>• Increases stool weight</td>
</tr>
<tr>
<td>• Mild laxative effect at higher intakes</td>
</tr>
<tr>
<td>• Reduces energy intake when substituted for normal starch in foods</td>
</tr>
<tr>
<td>• Reduces insulin response compared to normal starch/ carbohydrates</td>
</tr>
<tr>
<td>• Increase satiety response in the late post absorptive phase</td>
</tr>
<tr>
<td>• Increases Ca and Mg absorption</td>
</tr>
<tr>
<td>• Stimulates immune system</td>
</tr>
<tr>
<td>• Reduces risk factors related to large bowel cancer</td>
</tr>
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Biomarkers related to the study of gut health
Currently there is extensive debate on how to study the effects of selected nutrients on body functions and metabolism. The fact that the populations in Western...
countries grow significantly older than a few decades ago has aroused governments as well as health professionals to identify environmental factors that influence health. Reducing risk factors that are associated to disease has become an international target. Accordingly, producers of health foods and food supplements aim at placing disease risk reduction claims on the product packaging.

In this respect there is one generally recognized problem. The ingestion of the daily food will lead to the intake of a large number of compounds that may have some kind of bio-active effect. The latter may be an inhibition as well as an activation of a metabolic event. As such the effect of any dietary intervention is always the effect of a concerted action of all food components ingested. This makes the study of the effect of a single food component in terms of disease prevention a very difficult one. For example, if the study population would be composed of healthy individuals it would require maybe a few thousand of subjects and a study duration of 10–20 years or more to be able to detect a meaningful effect. For economical reasons this is mostly impossible. Related to this issue is the question whether you can make a healthy individual healthier anyway? To avoid such problems the focus generally is on the study of high risk populations, such as adenoma patients, in whom the recurrence of adenoma is studied as a marker of efficacy of nutritional compounds to reduce colorectal carcinogenesis. Also in this case it would require a high number of subjects and a long duration. Moreover, the question could also be raised as to whether such a patients group is representative enough to obtain results that are relevant to the general non-high risk population. This question has not been answered and the studies that have been done with adenoma patients generally have produced disappointing results. Other possibilities concern the study of animals that are exposed to selected carcinogens or inflammatory substances. Such studies can be done in short term and will help to unravel mechanistic aspects of the biochemical actions of the food compound studied. Using experimental gut carcinogenesis and gut inflammation models much data has been obtained on the possible role that products of fermentation may have on either reduction of cancer development and gut inflammation in these animals or recovery from it.

Again, also in this respect, the question is justified as to whether the observations done in animals are meaningful to the human situation. A last alternative is the use of human cell lines that are studied in vitro. On the one hand this comes much closer to influence on human tissues and organs, on the other hand many other influences that normally are present in the human body are eliminated in this situation. For example the concerted action of hormonal and neural influences and the bio-active effects of a many compounds that normally circulate with body fluids but are absent in vitro. There is one other aspect that should not be overlooked. Since the human gut, especially the colon, is very difficult to

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**Fig. 1.** Starch consumption in France declined significantly during the last 5 decades, most importantly by a reduced consumption of bread and potatoes. Accordingly also the calculated consumption of RS declined from 7–9 g/day initially to 3–7 g in recent years.
access for long-term study, science has to rely largely on animal models and in vitro work. It is in this light of these limitations that we have to deal with the current knowledge on gut health as specified in the sections that follow below.

**Butyrate actions and/or immunity effects?**

Important is also the recognition that the large bowel plays a significant role in our immune defence against disease. Research has shown that impairments in butyrate supply to colon cells induce gut atrophy and functional impairments, including reduced immune responses. In contrast, enhanced butyrate supply to the colon cells induces growth of the gut epithelium, gut cell differentiation and improvement of immune-surveillance. (Cummings, & Englyst, 1991; Roediger, 1990; Scheppach, 1994).

During the last decade it has been recognised that especially resistant starch induces a high butyrate and propionate production (Bird et al., 2000). Essential in this respect is that this production is also present in the lower part of the large bowel. It is this part (descending colon and rectum) where “faecal concentration” takes place and where the risks of developing colon cancer are a 1000 fold higher than in other parts of the intestine (Goodlad & Wright, 1995).

Butyrate is known to have beneficial effects on the reduction of risk factors involved in the etiology of colon cancer and adenoma development (Smith et al., 1998). Butyrate oxidation has been shown to make up for more than 70% of the oxygen consumption by the human colonic tissue (Roediger, 1980), indicating that butyrate is the prime energy substrate of the colonocyte.

Butyrate is an anti-neoplastic agent in vitro, and has been implicated in the protective effect of fibre in rodents (McIntyre, Gibson, & Young, 1993; Scheppach et al., 1995). Sodium butyrate (NaB) exerts an anti-proliferative activity on many cell types. It induces differentiation of colon carcinoma cell lines. It also has been observed to induce gene expression, to influence the rate of gene expression through its effects on post translational modifications, to induce apoptosis (natural programmed cell death) and to reverse the resistance of colonic cancer (uncontrolled growth) cells to apoptosis (Archer et al., 1998; Caderni, Luceri, Lancioni, Tessitore, & Dolarà, 1998; Smith, Yokoyama, & German, 1998; Velázquez, Howard, & Rombeau, 1996). Various lines of evidence suggest that the pathways of programmed cell death provide a means of protection against carcinogenesis by removing genetically damaged cells before they can give rise to pre-cancerous lesions. This has been reviewed in detail by Johnson (2001). In vitro experimental work further showed a decreased hydrogen peroxide induced DNA damage in human colon cancer cells following incubation with butyrate Rosignoli et al. (2001). Additionally it was shown colon in cancer cells (Caco-2) that butyrate improves intestinal barrier function by reducing its paracellular permeability after an epithelial damaging event Mariadason, Barkla, and Gibson (1997). SCFAs inhibited colon cancer cell (SW116) invasion. Effects were more potent for butyrate (Emenaker & Basson, 1998).

There is data suggesting that the ingestion of wheat bran promotes histone acetylation (most probably by the action of butyrate), a process involved in apoptosis induction in rat epithelial cells in vivo (Boffa, Lupton, Mariani et al., 1992).

Butyrate stimulates the immunogenicity of the cancer cells. The phenotype of the weakly immunogenic rat colon cancer PROb cells was modified with sodium butyrate. After a 4-day in vitro sodium butyrate treatment, the lymphokine-activated killer cell sensitivity, the expression of Major Histo compatibility Complex class I, and the intercellular adhesion molecule 1 of PROb cells, were increased in a dose-dependent manner (Perrin et al., 1994).

Perrin et al. (1994) also tested the efficacy of the immune factor interleukin 2 (IL-2) and sodium butyrate (NaB), alone or in combination, against experimental carcinomatosis, induced in rats by intraperitoneal injection of 2 × 10⁶ PROb colon carcinoma cells. IL-2/butyrate combination resulted in cases of complete cure of carcinomatosis with specific protection against PROb cells.

This complete regression of tumour masses may be attributed, to butyrate-induced effects on apoptosis (reintroduction) and an increase of the immunogenicity of the cancer cells.

Based on these experimental observations it is hypothesised that the ingestion of indigestible carbohydrates as an indirect source of butyrate to the large bowel may be beneficial in terms of reducing risk factors for colorectal cancer.

Highly interesting for health maintenance is also the specific action of butyrate to promote the growth and development of normal intestinal cells while inhibiting processes that lead to diseased and abnormal cells (Velazquez et al., 1996). When produced in higher amounts in the colon, butyrate may also have small systemic effects after absorption and circulation through the body. Accordingly it has been suggested in scientific literature that butyrate may not only protect against the initiation and development of large bowel cancer (Avivi-Green, Polak-Charcon, Madar, & Schwartz, 2000) but perhaps also breast (Heerd, Houston, Anthony, & Augenlicht, 1999) and prostate cancer (Ellerhorst et al., 1999).

Studies on the effect of butyrate, propionate and acetate 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 but also patients with colon cancer typically have low levels of butyrate in the gut (Chapman et al., 1994) and have a low rate of butyrate oxidation by the mucosa.

This may be related to a poor supply of fermentable substrate and a non-balanced intestinal flora, in which sulfate reducing bacteria are present in too large quantities. These bacteria produce increased luminal concentrations of mercaptans, sulfides and sulfites.

Mercaptans are known to inhibit the uptake of butyrate by the colon cells (Stein, Schröder, Milovic, & Caspar, 1995). Thus, 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 shortage of energy for optimal functioning and to a breakdown of adenine nucleotides along with the formation of free radicals through the xanthine oxidase reaction. Both a shortage of energy and the presence of free radicals will promote the inflammation and ultimately the necrosis process in the intestine (Jacobash, Schmiedl, Kruschewski, & Schmehl, 1999).

The fact that butyrate indeed plays an important role is confirmed by studies in which patients with inflammatory bowel disease received sodium butyrate enema. The supply of butyrate reduced inflammation related to enhanced butyrate availability after the ingestion of RS3 was most pronounced in controlled rat experiments (Butzner, Parmar, Bell, & Dalal, 1996; D’argenio et al., 1996). Recently Jacobasch et al. (1999) reviewed a number of studies while concluding that the synthesis of new laminin, the protein that makes up most of the basal membrane in the colon mucosa, is closely related to the onset of cell proliferation.

The latter is stimulated by butyrate obtained from RS3 consumption. Apoptosis (controlled cell death) was introduced more rapidly when butyrate was available. Accordingly colonic epithelial cells do have better regenerative properties when supplied with RS derived butyrate. A detailed review on mechanisms of diet related apoptosis is given by Johnson (2001). SCFA also are known to stimulate bowel motility, blood flow (Kamath, Philips, & Zinsmeister, 1988) and reduce enteral feeding associated diarrhoea by enhancing colonic water uptake (Bowling, Raimundo, Grimble, & Silk, 1993).

Of significant interest is also the very recent observation that there is a close relationship between the production of butyrate and enterolactone in the colon, as observed in an ileal cannulated and catheterised pig model ($\nu = 4$) (Bach Knudsen, Serena, Glitso, & Adlercreutz, 2001). Enterolactone is a mammalian lignan that is produced by the intestinal flora, in the upper part of the colon, from lignans (a class of phytoestrogens) that are present in certain plant foods like whole grain, especially rye (Glitso et al., 2000). Lignans have been described to protect against a number of Western diseases through improving the host’s health (Adlercreutz, 1990). This new observation suggests that modulations of either intestinal bacterial metabolism and/or intestinal flora after the consumption of well fermentable dietary fibers most probably leads to an induction of enterolactone production. When confirmed in other studies, the potential inclusion of resistant starch as ingredient in whole grain products may become very attractive.

Observations as outlined above have prompted the food industry to give more focus on dietary fibres that lead to a high butyrate production in a large segment of the colon. In this respect, Baghurst et al. (1996) indicated that “for the food industry, one potential advantage of resistant starch, over non-starch polysaccharides as agent for “fibre like” activity, is the much greater potential to increase intake in the community, through the development of new high resistant starch products.” Baghurst et al. (1996) further commented, that “for some core starch foods such as breads, it has already been possible to increase resistant starch consumption by some 8–10 fold. A similar increase in non-starch polysaccharides (NSP) content is very difficult to achieve in whole foods. If resistant starch proves to be as physiologically effective on a weight for weight basis as NSP, then the health potential of this “new” food component will be high”.

A new type of natural RS3 with potential gut health properties

From the above mentioned observations it appears that the amount of butyrate produced in a relatively large segment of the colon is of significant importance. The average ratio of NSP derived acetate / propionate and butyrate is 60:25:10 respectively (Cummings, 1981). A substantial number of studies have shown that the short chain fatty acid profile resulting from RS fermentation differs from that of NSP fermentation. RS fermentation generally results in a relatively large butyrate production in the order of 20–28 mol% compared to about 10–15 mol% for NSP. This has been shown both in vitro and in vivo thus, not the NSF fraction itself but the quantity of fermentable starch, reaching the colon, is a strong determinant for the quantity of butyrate being produced (Perrin, 2001). Recently a new type of natural highly crystalline RS3 (Actistar\textsuperscript{®}, (Act*-RS3)) has been developed from maltodextrins as starting material. (US Patent 6,043,229 (2000). Act*-RS3 has a relatively low average molecular weight and a much
homogeneous molecular weight distribution than RS products based on high amylose starch. Due to raw material choice and production process, it has a very neutral taste. Regular shape and low particle size are the reason for a pleasant mouth feel without any sandiness. It can be easily dispersed in cold and hot fluids without lumping and gelling tendency respectively. The physiological properties seem to be a new challenge for the food industry in their attempts to target the Gut health market. A number of laboratory trials have given insight in the technical as well as functional properties of Act*-RS3® and its metabolism.

**SCFA production**

The molar proportion of SCFA produced during fermentation depends on the quality of the RS. That is, the extent to which the RS is accessible to the microflora.

RS3 is a very good fermentable substrate. The supplementation of RS3 to food may more than double in the luminal concentrations of butyrate and propionate (Kleesen et al., 1997), which may be essential in terms of sustained effects on colonic metabolism.

An *in vitro* fermentation assay of ACT*-RS3® has been performed in a batch system with human faeces according the methodology described by Barry et al. (1995). Its fermentation pattern was shown to be complete and rapid, resulting in a dose dependent increase of the total SCFA’s (Fig. 2) and a concomitant drop in pH. Butyrate represents 21–28 mol% of total SCFAs, (for comparison, most NSP’s result in 10–15% butyrate).

Arrigoni, Rochat, and Amado (2001) used an *in vitro* batch system with pre-digested substrates, as described by Lebet, Arrigoni, and Amado (1998). They compared various oligosaccharides and RS.

Compared to indigestible short-chain oligosaccharides (fructo-oligosaccharides, xylo-oligosaccharides) the fermentation of RS is relatively slow. The production of hydrogen after RS intake is lower and the production of butyrate was significantly higher. According to the authors this may be due to the specific presence of partially crystalline retrograded amylose in the product (RS3). When compared to retrograded debranched high amylose corn starch (Cristalean, Opta Foods) and RS2 (thermally modified granular high amylose corn starch, Novelose 240, National Starch, USA), ACT*-RS3® resulted in a significantly faster substrate disappearance, higher total SCFA and butyrate production at 8 hrs of incubation. (Figs. 3 and 4 and Table 3) (Arrigoni et al., 1999, 2001). This indicates that the product is easier fermentable by the colonic microflora. Interestingly, a study by Martin, Dumon, Lecannu, and Champ (2000) in pigs* suggested that butyrate produced from RS3 is more distally fermented in the colon and could be more beneficial for a healthy colon than raw potato starch.

![Starch consumption in western countries](image)

**Fig. 2.** Production of butyrate from wheat bran, pectin and Act*-RS3. The SCFA production was measured in an *in vitro* batch system with human faeces.
(RS2) (* pigs are very close to humans in terms of the digestive system).

**Immunogenicity**

Experimental studies using animal models that are assumed to be most representative to study the effects of nutrients on the initiation and progression of colon cancer have shown that Act*-RS3* consumption induces the rejection of pre-cancerous cells (aberrant crypt foci) via direct actions, most probably through its butyrate delivery. It has been suggested that this effect is further indirectly supported by an improved immunogenicity of the colon cells as well as a modulation of blood immune parameters (Menaut et al., 1998; Perrin et al., 2001; Pierre et al., 1997).

**Digestibility**

In *in vivo* human studies using ileostomy patients as model have shown that the product contains 54% (DS 60%) of type 3 RS that reaches the colon for fermentation. The ileostomy model was earlier shown to give reliable results for *in vivo* measurement of RS (Langkilde, Philipsson, Andersson, & Brouns, 2001). The other fraction of 46% is digestible and absorbable carbohydrate that will contribute to daily carbohydrate intake (Langkilde 2001). This observation implicates that consumption will reduce caloric intake as well as insulinenia when compared to the consumption of normal starch. Accordingly, the inclusion of RS3 in foods may be suitable for a wide variety of food products designed for overweight individuals and type II diabetics.

**Digestive tolerance**

In order to promote an increased regular consumption of type 3 RS consumers desire that a reasonable daily consumption of 20–30 g will not induce digestive side effects that may disturb the quality of life. Inherent to increased fermentation of most carbohydrates is the increased flatus production. With rapidly fermentable carbohydrates this may also result in less desired bloating of the gastrointestinal tract. In this respect a recent study on digestive tolerance of RS3 was conducted in healthy volunteers (n=41) at the Nutritional Biosciences Department of the Salford University (UK) (Lee, Storey, Bornet, & Brouns, in press). This study evaluated the effects of an acute daily dose of ACT*-RS3 (0, 20, 40, 60, 80, 100 and 120 g presented in random order). The second objective was to study the effect of gradually, increasing the dosages over a period of 21 days with the goal to finally reach a threshold dose equal to the dose that was maximally tolerated without disturbing digestive problems in the acute intake condition.

Symptoms were considered to be: nausea, bloating, borborygmni, colic, flatus, diarrhoea in such a way that the subject judged these to be disturbing. With the acute dose, three subjects had a threshold tolerance of 80 g, 8 subject of 100 g and 30 subject of 120 g resp. Regression analysis showed that there was no significant increase in mean symptom scores for any symptom. Mean numbers of toilet visits to pass faeces of any consistency: normal, watery or hard faeces, following consumption of increasing doses of RS3 did not change either.

From the chronic consumption data, it could be concluded that repeated ingestion of RS3 did not cause any increase in the number of subjects experiencing severe...
symptoms, compared to the acute condition. Accordingly, the authors concluded that up to 60 g RS per day (equivalent to 120 g ACT*RS3) was well tolerated by subjects but a mild laxative effect at 60 g was suggested (Lee et al., in press).

Food application characteristics

Low hygroscopicity, low water binding capacity and very low and stable suspension viscosity enable an easy incorporation in numerous food recipes. Under most normal processing conditions the resistant retrograded structure is stable. The use of ACT*-RS3 in bread, biscuits, or pasteurised milk products resulted in a 95–100% recovery of RS structures. This means that substantial amounts of crystalline structures (RS) are recovered after heat treatment of food preparations. Even under very harsh conditions such as UHT (137°C for 5 s) about 2/3 of the retrograded structure is retained. Possible inclusion levels of RS3 in foods and drinks are presented in Table 2.

Summary/conclusions

Food consumption studies show that RS consumption has declined over the last decades. There are reasons to suggest that increasing the RS content in the daily diet will be of benefit for the maintenance of gut health and the reduction of risk factors associated with the development of intestinal inflammation and colorectal cancer. A significant resistant starch consumption and its related fermentation pattern may act on the colon in several ways:

1. stimulation of the local immune system (GALT)
2. modulation of blood immune parameters
3. modulation of DNA synthesis and repair by butyrate
4. stimulation of normal cell growth by butyrate
5. inhibition of abnormal cell growth and development by butyrate
6. promotion of recovery from epithelial inflammation.

A new type RS3 has been developed with excellent technical and functional properties for application in food:

- contains 54% resistant starch (60%DS)
- high butyrate as well as substantial propionate production in colon.
- low glycemic and insulinemic response.
- remarkably good digestive tolerance
- non-starchy taste, no sandy mouth feel.

Future research with foods that have significant inclusion levels of RS3 should focus on validating the promising results from experimental animal studies in a human situation. Seen the fact that different types of RS may have a different fermentation profile and butyrate production, it is important that results are obtained especially on RS3, the resistant starch type mostly consumed by humans and most distally fermented in the colon in animal experiments (Martin et al., 2000).

References


<table>
<thead>
<tr>
<th>Table 2. RS III-enriched products: inclusion levels tested without taste effects compared with non-enriched standard products (*note: in vivo resistant starch content of ACTISTAR amounts 54% (60%DS))</th>
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</thead>
<tbody>
<tr>
<td>Proposed % ACT-RS3&lt;sup&gt;III&lt;/sup&gt; inclusion*</td>
</tr>
<tr>
<td>Cereal-based products</td>
</tr>
<tr>
<td>Bread</td>
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<tr>
<td>Rusk</td>
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<td>Breakfast cereals</td>
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<td>Muesli</td>
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<tr>
<td>Pasta</td>
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<tr>
<td>Dessert and biscuits</td>
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<tr>
<td>Savory biscuits and cookies</td>
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<tr>
<td>Vegetables</td>
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<tr>
<td>Soup</td>
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<tr>
<td>Dairy products</td>
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<tr>
<td>Milk</td>
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<td>Yoghurt</td>
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<th>Table 3. Molar proportion of the three main short chain fatty acids produced from pre-digested resistant starch preparations after 24 h of in vitro fermentation (mol%)</th>
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<tbody>
<tr>
<td>Acetate</td>
</tr>
<tr>
<td>RS 2</td>
</tr>
<tr>
<td>RS 3a</td>
</tr>
<tr>
<td>RS 3b</td>
</tr>
</tbody>
</table>
Champ, M., Langkilde, A. M., Brouns, F., Kettlitz, B., & Le Bail-Collet, Y. Advances in dietary fibre characteristics. 