9 Resistant Starch and Starch-Derived Oligosaccharides as Prebiotics

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Dietary fiber has long been recommended as part of a healthy diet based on the observations made by Burkitt and Trowell (1975). Since then, epidemiological evidence has consistently shown that populations consuming higher levels of foods containing fiber have decreased risk of a variety of chronic health disorders such as cardiovascular disease, type II diabetes, and certain cancers. Average fiber intake in the United States is approximately 13 g/day for women and 18 g/day for men (National Academy of Sciences, 2006). The FDA recommends a minimum of 20–35 g/day for a healthy adult depending on calorific intake. In many EU countries including France, Germany and the UK (see *Figure 9.1*), fiber intakes are much lower than authorities recommend for men and women (Buttriss and Stokes, 2008; Gray, 2006). Thus, there is a need to increase fiber consumption and many newly isolated or developed fibers can easily be added to beverages and processed foods. The reasons for such low compliance is somewhat complex, however the most basic rationale for not consuming fiber-rich foods is perceived bad taste and mouthfeel and the availability of conventional food items containing fiber.

Dietary fibers confer a wide range of health effects, from alleviation of constipation to reduction of cholesterol (Buttriss and Stokes, 2008). The physiological effects of dietary fibers in humans depend on the physico-chemical properties of fiber (viscosity, fermentability, bulking properties) and on the human gastro-intestinal (GI) tract (gut microbiotia, GI transit time). A specific subset of dietary fibers, so called prebiotics, convey health benefits by selectively stimulating the growth and/or activity of one or a limited number of bacteria like bifidobacteria and lactobacilli in the colon (Gibson and Roberfroid, 1995).

There is considerable industry and public interest in the capacity of foods and food components to promote health and lower risk of non-infectious

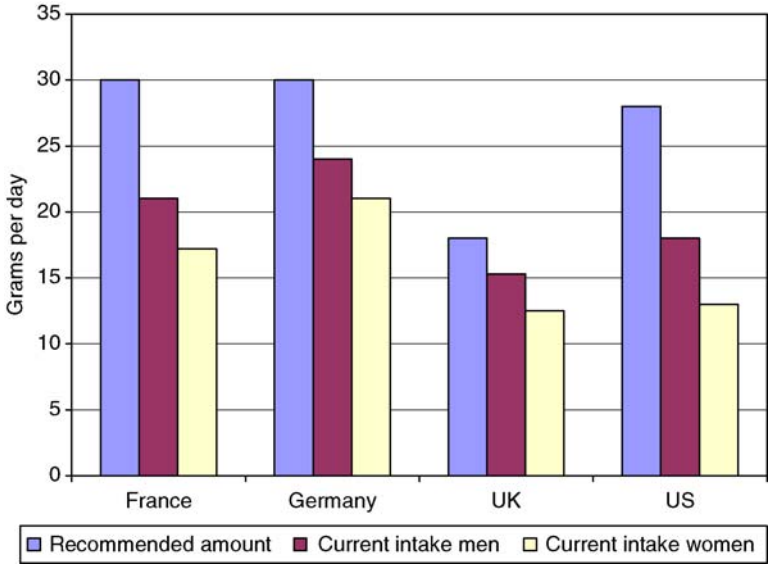


Figure 9.1
Daily intake of fibers.

diseases related to diet and lifestyle. Industry is thus challenged to develop fibers that can overcome the problems with stability under manufacturing conditions, functionality in various food systems, and taste in order to give the consumer more options when it comes to getting fiber in their diet. Newly derived starch fibers meet these requirements. In this chapter, prebiotic properties of newly derived fibers from maize and other starches will be reviewed with a specific focus on PROMITOR™ fibers, which were designed for optimal taste and texture and have prebiotic properties. The PROMITOR™ line includes a Soluble Gluco Fiber (PROMITOR™ Soluble Corn Fiber in US) and an insoluble Resistant Starch that are classified as food ingredients.

9.1 Introduction

The human gut microbiota constitutes a dynamic and ecologically diverse environment. The large intestine is by far the most heavily colonized region of the digestive tract, with up to 10^{12} bacteria for every gram of gut contents containing more than 400 different species of bacteria. The number of bacteria in the colon outnumber (10-fold) the number of human cells making it a very powerful target for nutritional interventions. Through the process of fermentation, colonic

bacteria are able to produce a wide range of compounds that have both positive and negative effects on the gut physiology as well as other systemic influences. For instance, certain microbial populations present in the gut provide an efficient barrier to invading pathogens (Macfarlane, 2008). Competition for nutrients and ecological niches, production of antimicrobial compounds, lowering of intestinal pH through production of short chain fatty acids and stimulation of the immune system play a role in limiting the ability of pathogens to colonize the gut and potentially cause disease. Many of these microbiota-associated activities have a direct impact on host health. While prebiotics are selectively interacting with the intestinal microbiota, they are being fermented by the bacteria into many different metabolites. As the composition of the microbiota is modified, the types of fermentation metabolites into which prebiotic substrates are converted are also modified. Some of these metabolites are utilized by the cells lining the intestine, while others are absorbed into the blood of the host and pass the blood barrier to enter the systemic body space, where they interact with many physiological processes in all vital organs and peripheral tissues of the host (Lenoir-Wijnkoop et al., 2007).

9.2 Resistant Starches

Resistant starches are defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1997). There are four main groups of resistant starches: RS1, RS2, RS3 and RS4. RS1 is physically inaccessible starch (i.e., starch in whole grains), RS2 is granular starch i.e., starch in green bananas), RS3 is retrograded starch (i.e., starch in cooked and cooled potatoes) and RS4 is a chemically-modified starch (i.e., an esterified starch). PROMITOR™ Resistant Starch is classified as a type 3 resistant starch.

9.2.1 Introduction to a Type 3 Resistant Starch

9.2.1.1 Regulatory Status

Resistant starches occur naturally in many foods and thus have been safely consumed across the globe for years. PROMITOR™ Resistant Starch is a food ingredient in the US and EU and can be labeled as "Resistant Starch,"

"Starch," "Maize Starch" or "Corn starch." It is a non genetically-modified source of dietary fiber which enables, where relevant regulatory conditions are met; the use of fiber nutrient content claims (contains fiber, source of fiber, high fiber).

The European definition of dietary fiber published in Directive 2008/100/EC recognizes as dietary fiber, carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

- edible carbohydrate polymers naturally occurring in the food as consumed
- edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence
- edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence

RS meets the European definition and can be incorporated into food products to meet the Regulation (EC) 1924/2006 that requires at least 3 g of fiber per 100 g or at least 1.5 g per 100 kcal for the nutrition claim "source of fiber"; and at least 6 g of fiber per 100 g or at least 3 g per 100 kcal for the nutrition claim "high in fiber." Levels necessary for nutrient content claims in the US are 2.5 g fiber/serving for a "good source" claim and 5.0 g fiber/ serving for an "excellent source" claim.

9.2.1.2 Dietary Fiber Content

PROMITOR™ Resistant Starch has an analysis of approximately 60–75% total dietary fiber per AOAC method 991.43. This AOAC method works well for fibers that are insoluble or nearly insoluble.

9.2.1.3 Calorific Value

RS has a calorific value of 1.70 kcal/g, as calculated using standard practices of subtracting the percent fiber content as analyzed by AOAC 991.43 from total carbohydrates. This is the calorific declaration used on specification sheets in the US, as specified by US regulations for insoluble fibers. EU calorific value will be 2 kcal/g as per Commission Directive 2008/100/EC.

The true metabolizable energy (TME) content was also determined in an *in vivo* avian model at the University of Illinois (Knapp et al., 2008). The model is a more precise model for calorific determination than *in vitro* models and collection of urine and feces is easier and often more precise than in humans. This method, using bomb calorimetry, determined the calorific value of RS to be in the range of 1.7–2.0 kcal. This suggests that either a small portion of the RS is digested, or that fermentation products generated in the colon adds a small fraction of calories. Fermentation is a more likely hypothesis based on human glycemic response data. The glycemic response for RS is approximately 10% that of a maltodextrin control (Kendall et al., 2008). Since maltodextrin and RS are both glucose polymers, a glycemic response of 10% suggests that the remaining 90% of RS remains undigested and enters the colon. Thus, it is likely the majority of the calories from RS may actually come from fermentation metabolites.

9.2.1.4 Digestive Tolerance

Type 3 RS was shown to be well tolerated up to doses as high as 45 g/d (Bouhnik et al., 2004). Stewart et al. (2008, 2009) conducted a study in which subjects (n = 20, 10 men, 10 women) consumed 12 g fiber/d for 14 consecutive days. Subjects were asked to report gastrointestinal symptoms (cramping, bloating, stomach noise, flatulence) on a 10 point scale (1 = minimal symptoms, 10 = severe symptoms). RS was shown to be well tolerated (Stewart et al., 2009). In a dose-response study, tolerance was assessed in 22 healthy volunteers consuming three different doses of RS (5 g, 15 g, 25 g) in acute conditions after an overnight fast (Kendall et al., 2009). Subjects were asked to report gastrointestinal symptoms (belching, bloating, flatulence, nausea and diarrhoea) on a 100 mm VAS scale at 0, 15, 30, 45, 60, 90 and 120 min after consumption. RS was shown to be well tolerated in these conditions.

9.2.1.5 Applications

RS acts as an insoluble fiber that can be added to baked products, cereal products or snacks, providing them with higher fiber levels as well as other health benefits. It is one of the most stable resistant starches on the market (unpublished data) and so can be used in extruded or sheared and baked products with less loss of fiber during processing. As a result the final product has a high fiber content while

offering a range of health benefits. With only 1.70 kcal/g, it can also be used in low calorie products reducing both calories and carbohydrates when replacing flour or other cereal-based ingredients.

Applications for RS include puffed or sheeted snacks, chips, extruded breakfast cereals, pasta, muffins, cookies and biscuits, crackers, frozen dough, breads.

It can be used as a partial replacement for flour in bakery products that exhibit characteristics comparable to those achieved using conventional wheat flour (e.g., cookie spread, golden brown color, pleasant aroma, surface cracking). Thanks to its low water holding property, it also does not affect height and spread management of biscuits, cookies or other baked goods.

RS enhances crispiness of cookies and crackers as well as the surface of baked sheeted crackers and extruded products. Furthermore, the induced reduction of water activity and moisture content, enhance sensory characteristics as well as the shelf life of goods. Notably, it tends to decrease bulk density, improving expansion in extruded cereals and snacks. In fried snacks, fat uptake may be reduced by up to 25% when RS is used, helping to meet "high/rich in fiber" claims.

Moreover, with a thermal stability as high as 150°C, it will retain more fiber content and structure than other resistant starches, which start to break down below 120°C.

9.2.2 Prebiotic Properties of Various Resistant Starch Products (RS2 and RS3)

9.2.2.1 Effect on Microbiota Modulation

The ability of RS to favor growth of bifidobacteria and lactobacilli within the gut flora has been assessed *in vitro*, in animal models and in humans.

In a study Le Blay et al. (2003) showed the prebiotic properties of RS2. Eighteen rats were fed a low-fiber diet (Basal) or the same diet containing raw potato starch (RS2) (9%) or short-chain FOS (9%) for 14 days. Changes in wet-content weights, bacterial populations and metabolites were investigated in the caecum, proximal, distal colon and feces. Both substrates exerted a prebiotic effect compared with the Basal diet. All bacteriological analysis were performed within 2 h after sampling. Samples were diluted and dilution were applied on plates using both unselective and selective media. After incubation, single colonies were counted. FOS increased lactic acid-producing bacteria throughout the caecocolon and in feces, whereas the effect of RS2 was limited to the caecum and proximal

colon. As compared with RS2, FOS doubled the pool of caecal fermentation products, while the situation was just the opposite distally. This difference was mainly because of the anatomical distribution of lactate, which accumulated in the caecum with FOS and in the distal colon with RS2. Feces reflected these impacts only partly, showing the prebiotic effect of FOS and the metabolite increase induced by RS2. In conclusion, this study demonstrates that FOS and RS2 exert complementary effects and combined ingestion could be beneficial by providing health-promoting effects throughout the colon.

Brown et al. (1997) also observed prebiotic effects in 12 young male pigs fed with high amylose cornstarch diet (RS2). Starch provided 50% of total daily energy either as a low amylose cornstarch or as a high amylose (amylomaize) cornstarch. Fecal output, fecal concentrations and excretion of total SCFA (notably propionate and butyrate), fecal culture-based bifidobacteria counts (expressed per gram of wet feces) and their daily fecal excretion were higher when pigs were fed the high amylose cornstarch.

Similar prebiotic effects have been reported for retrograded RS (RS3) in several animal models. Dongowski et al. (2005) and Jacobasch et al. (2006) have shown in rats fed with RS3 that the growth of bifidobacteria and the production of SCFA were increased throughout the digestive tract, favoring thus a decrease of the pH in the caecum, colon and feces.

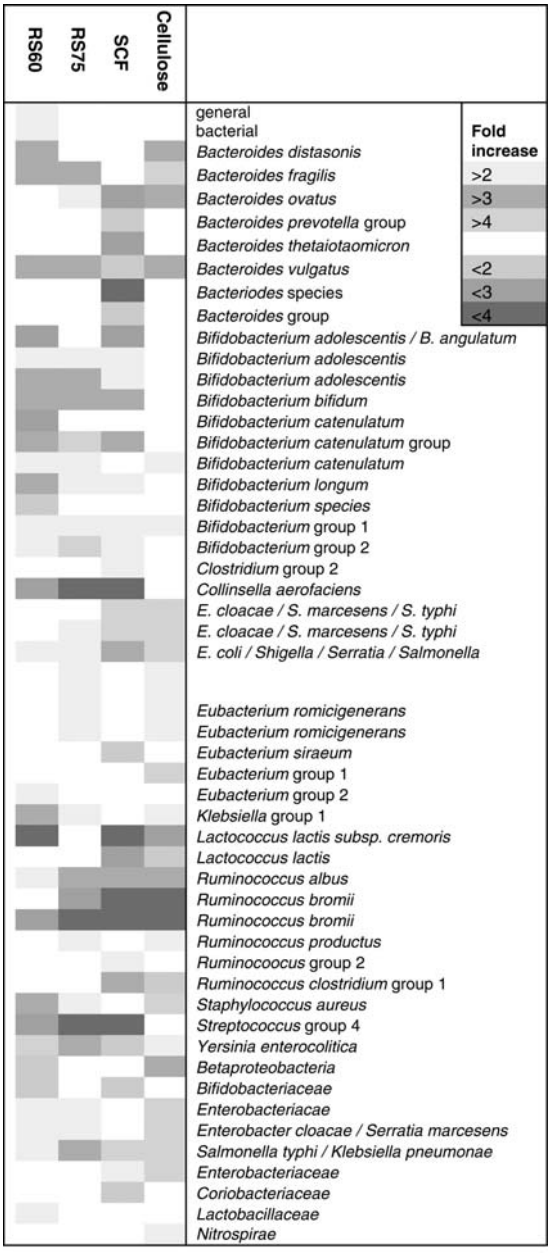
Using another model of human flora-inoculated gnotobiotic rats (HFA), colonized with microbiotas from UK or Italian donors, Silvi et al. (1999) looked at the prebiotic effects of a RS3. Consumption of this RS3 (15 g/100 g diet) resulted in significant changes in both the UK and Italian flora-associated rats: numbers of lactobacilli and bifidobacteria were increased 10–100-fold, and there was a concomitant decrease in enterobacteria when compared with sucrose-fed rats (control). The induced changes in caecal microbiota of both HFA rat groups were reflected in changes in bacterial enzyme activities and caecal ammonia concentration. This RS3 markedly increased the proportion of n-butyric acid in both rat groups, lowered caecal ammonia concentration, caecal pH and beta-glucuronidase activity.

The prebiotic properties of RS3 have also been demonstrated in humans (Bouhnik et al., 2004). First, this study determined the bifidogenic properties of a RS3 at 10 g/d; Second, the dose-response relationship of the bifidogenic effects of this RS3 at doses ranging from 2.5 to 10 g/d in comparison with a placebo were assessed. Faecal samples were diluted and dilutions were applied on plate using different selective media. RS3 was shown to be bifidogenic at a dose of 10 g/d. However, bacteria counts increased at doses of 5 and 7.5 g/d, but

decreased at doses of 2.5 and 10 g/d. Thus, no firm conclusions could be drawn from this study on bifidogenic properties of RS3 in humans, however the authors observed that only a low baseline bifidobacteria count was significantly associated with a bifidogenic response to treatment. This observation was recently corroborated by Roberfroid who highlighted that the baseline level of bifidobacteria together with the time of exposure to a prebiotic are more determinant factors than the amount of prebiotics consumed to assess the potential prebiotic properties of a fiber (Roberfroid, 2007). The lack of bifidogenic effect of RS3 in this study at 2.5 and 10 g/d in phase 2 might be primarily linked to an elevated baseline bifidobacteria count in the groups of volunteers.

In this respect, a recent *in vitro* study also showed that the RS3 crystalline polymorphism can impact the RS fermentability by human gut microbiota as well as the short chain fatty acids production. Human fecal pH-controlled batch cultures showed that RS induces an ecological shift in the colonic microbiota, with polymorph B being much more efficient in inducing *Bifidobacterium* spp. growth than polymorphism A. Interestingly, polymorph B also induced higher butyrate production to levels of 0.79 mM (Lesmes et al., 2008). Type-A crystalline polymorph is found in typical cereal starch granules while the type-B polymorph is found in potato and high amylose cereal starch granules. The A polymorph has a much lower water content in the crystal lattice compared to the B polymorph.

PROMITOR™ Resistant Starch has shown to be a potential prebiotic in *in vitro* studies at TNO (Maathuis et al., 2008). The aim of the study was to investigate the effect of newly developed maize-based fibers on the activity and composition of the microbiota in the colon. The tested fibers were glucose-based and have variable structures including two resistant starch preparations. The fibers were pre-digested, mono- and di-saccharides were removed, and the residual polymer was used to assess the production of microbial metabolites and changes in composition of the microbiota using a dynamic, validated, *in vitro* model of the large intestine (TIM-2). Microbial metabolite analysis showed an increase in health-promoting metabolites (short-chain fatty acids) and a reduction in toxic metabolites from protein fermentation compared to the poorly-fermentable control (cellulose). The lactate production was also relatively low, indicating that it is slowly fermented. This may contribute to its excellent tolerance and extend its health benefits throughout the entire large intestine. Using microarray technology to compare multiple species and groups of colonic microbiota, RS was found to promote the growth of beneficial bacteria such as bifidobacteria and lactobacilli (*Figure 9.2*). Further studies are underway to determine if these *in vitro* effects are also seen *in vivo*.



SGF: Soluble Gluco Fibre/Soluble Corn Fibre; RS 60: Resistant Starch 60%; RS 75: Resistant starch 75%

Figure 9.2

Use of the microarray technology to evaluate the effects of PROMITOR™ fibers on multiple species and groups of colonic microbito.

Based on the above-mentioned studies, it can be reasonably concluded that RS2 and RS3 have prebiotic activity. The mechanisms involved in promotion of bifidobacteria/lactobacilli growth may be different than those observed for the other prebiotics (e.g., FOS or inulin). A study with FOS and high amylose starch (RS2) in pigs fed a diet based on human foods showed that both raised fecal bifidobacteria numbers by approximately equal amounts when fed separately. When fed together there was an increase that exceeded the individual increases, suggesting that they operate by different mechanisms (Brown et al., 1997). If FOS acts as a metabolic substrate for bifidobacteria and lactobacilli, RS2 seems to function differently. Indeed, *in vitro* studies showed on one hand that pure bifidobacteria strains have limited capacity to use RS2 as a substrate (Topping et al., 2003); on the other hand, they also showed physical adhesion of several bifidobacteria species to RS2 (Topping et al., 2003), suggesting thus that the prebiotic properties of RS2 may be linked to its ability to confer physical protection on the bifidobacteria/lactobacilli throughout the upper digestive tract. The same reasoning may apply to RS3, as Brouns et al. (2007) have shown that breast-fed babies' microbiota, mainly composed of bifidobacteria was unable to use RS3 as a substrate, though several animal and human studies have shown prebiotic effects of RS3.

Nevertheless, it seems that a limited number of bifidobacteria strains has the ability to use RS2 as a substrate as shown in the study of Crittenden et al. (2001). In this study, 40 *Bifidobacterium* strains were examined to complement RS in a synbiotic yogurt. Only *B. lactis* Lafti B94 possessed all of the required characteristics. This isolate was the only one able to hydrolyze Hi-maize (RS2), to survive well in conditions simulating passage through the gastrointestinal tract and to possess technological properties suitable for yogurt manufacture. *Bifidobacterium lactis* Lafti B94 survived without substantial loss of viability in synbiotic yogurt containing Hi-maize during storage at 4°C for 6 weeks. In this study, RS2 was seen as a good complementary prebiotic ingredient for new synbiotic functional food products.

9.2.2.2 Health Benefits Associated with Prebiotic Properties of Resistant Starch

Several health benefits have been linked to prebiotics, notably to FOS, GOS and inulin. One of these benefits is enhancement of the body's natural immune defenses (Schley and Field, 2002; Vos et al., 2007). This effect is primarily

localized to immune defenses in the gut, such as the gut-associated lymphoid tissue, however some studies have shown systemic effects. The precise mechanisms by which prebiotics exert their immune effects are unclear; whether it is through changes in the microbial population, through fermentation metabolites (i.e., SCFA) or through direct interaction of the prebiotics with mucosal membrane receptors. There have been very few investigations into the immune-modulating effects of resistant starch. Preliminary data from a recent study including PROMITOR™ Resistant Starch demonstrates a potential immune-modulating response in an animal model of inflammatory bowel disease. Animals supplemented with Resistant Starch had fewer macroscopic lesions in the gut and a reduction in the size of mesenteric lymph nodes (unpublished data) as compared to animals without Resistant Starch in the diet.

Another benefit seen with fermentable and prebiotic dietary fibers is enhancement of mineral absorption and/or increase in bone mineralization and bone density. There are numerous hypothesized mechanisms by which fermentable fibers may alter mineral absorption and impact bone density, a few of which include increased solubility of minerals, increased gastrointestinal surface area for absorption (by means of SCFA production), and alteration of the microbial population (Scholz-Ahrens et al., 2007). Many of the studies surrounding mineral absorption and bone formation have included prebiotics, such as inulin and FOS. However, RS has also been reported to enhance the ileal absorption of a number of minerals in rats and humans. Lopez et al. (2001) and Younes et al. (2001) reported an increased absorption of calcium, magnesium, zinc, iron, and copper in rats fed RS2-rich diets. Similar preliminary results have been seen in an animal study investigating PROMITOR™ Resistant Starch where it was shown to increase femur calcium concentration after 12 weeks of supplementation (Martin et al., 2009).

9.2.2.3 Gut Health Biomarkers

It has been shown that RS has health-promoting actions on the colonic microbiota beyond the prebiotic effect. For instance, studies in children with cholera-induced diarrhoea having consumed RS (high-amylose starch) plus the rehydration therapy have shown major reduction in fluid loss and a halving of time to recovery (Ramakrishna et al., 2000, 2008). This study has been replicated in babies with other forms of infectious diarrhoea where it was shown that both RS (as green bananas) and non-starch polysaccharides (NSP) facilitated recovery and improved intestinal permeability (Rabbani et al., 2001). Accelerated recovery

from infectious diarrhoea has also been shown in animals. A specific microorganism, *Brachyspira hyodysenteriae*, causes substantial economic losses in the commercial pig breeding industry through morbidity and mortality in the weaning period. The effect is expressed as diarrhoeal disease on the introduction of solid food. Feeding with cooked rice, an established source of RS (Marsono et al., 1993), lowers the incidence and severity of disease with a consequent reduction in mortality (Hampson et al., 2000). Part of the benefit seems to be due to increased fluid absorption through greater SCFA production, as these acids stimulate the uptake of water and cations (Na^+, K^+, and Ca^{2+}), particularly in the proximal colon. Several studies in humans (*Table 9.1*) as well as in animals (*Table 9.2*) have shown the ability of RS to enhance production of SCFA in the ceacum and throughout the colon. This outcome is an obvious mechanism for reversing diarrhoea-induced fluid loss. SCFA also appear to modulate the muscular activity of the large bowel and to promote the flow of blood through the viscera; both these actions could assist in lowering the severity of diarrhoea. In addition to these well-documented effects, it is possible that RS could limit the viability of the cholera organism in the gut. It may be hypothesized that the bacteria could adhere to the starch granules, very much in the same way as bifidobacteria, and thus be removed from the site of action (Topping et al., 2003). The production of SCFA (Cummings et al., 1996; Heijnen et al., 1998; Jenkins et al., 1998; Muir et al., 2004), the reduction of colonic pH (Birkett et al., 1996; Heijnen et al., 1998; Phillips et al., 1995) together with a beneficial change in microbiota metabolism pattern induced by RS intake (*Tables 9.1* and *9.2*) are further means of biocontrol for pathogens and potential pathogens.

Diet and its interaction with the gut microbiota, and reduced protection from the microbiota with age are likely contributory factors to colorectal cancer (CRC). Genotoxic or carcinogenic metabolites produced or activated by the gut microbiota provide a diversity of environmental insults, which play a role in the initial stages of cancer (Tuohy et al., 2005). There is considerable interest in using microbiota-modulating tools such as prebiotics (Burns and Rowland, 2000) or RS (Cassidy et al., 1994) to protect against colonic tumor development or to maintain a good colonic physiology.

To date, several human studies have examined the effect of RS on human colonic function (*Table 9.1*). These intervention studies have evaluated different types of RS, in combinations or alone, at different amounts. Some studies have mimicked human diets in that they have used a range of food-based sources, whereas others have used single manufactured forms.

Table 9.1

Human studies investigating the effects of RS intake on gut health biomarkers (*Cont'd* p. 272)

Sample size and study length	Intervention	Parameters measured / outcome	Authors
14 healthy subjects - 4 weeks	- 45g Hylon VII (62% RS) to a standard diet - standard diet low in RS	Cytotoxicity of fecal water on colon cell line: – Cell proliferation in rectal biopsies: – Fecal output: ++ Fecal SCFA: ++ Fecal bile acids: ++ Fecal secondary bile acids: –	Van Munster et al, 1994
11 healthy subjects in a randomized controlled cross-over study - 3 weeks	- High RS diet (raw banana flour + unprocessed wheat seeds + high-amylose maize cornbread, 26 to 50g/d) - Low RS diet (cooked banana flour + processed wheat seeds + low-amylose maize cornbread, 3 to 8g/d)	Butyrate: ++ Faecal pH: – Faecal excretion of NSP: ++ Feacal ammonia: – Fecal output: ++	Phillips et al, 1995
11 subjects in a randomized controlled cross-over study - 3 weeks	- High RS diet (≈40g/d, RS1, RS2, RS3 mix) - Low RS diet (5g/d)	Faecal nitrogen excretion: ++ Feacal ammonia: – Faecal phenols: – Faecal pH: –	Birkett et al, 1996
12 healthy subjects in a controlled cross-over study - 15d periods	- 17-30g RS2 diets - potato and banana starch - 17-30g RS3 diets - retrograded maize and wheat starch - RS-free diet	Faecal output: ++ Faecal SCFA: ++ Faecal excretion of NSP: ++ Fecal nitrogen excretion: ++ Transit time: –	Cummings et al , 1996

Table 9.1

Sample size and study length	Intervention	Parameters measured / outcome	Authors
23 hypertriglyceridemic subjects in a randomized controlled cross-over study - 4 weeks	- High amylose maize starch diet (RS:17-25g/d) - Oat bran: low-RS diet	Faecal pH: – Faecal SCFA: ++, butyrate Secondary faecal bile acids excretion: –	Heijnen et al, 1998
12 subjects in a controlled, cross-over study - 4 weeks	- Controlled basal diet enriched in RS (amylomaize starch, 55g/d RS) - Controlled basal RS-free diet	Faecal output: ++ Faecal SCFA: no effect β-glucuronidase: – Faecal bile acids excretion: – Faecal secondary bile acid: – Feacal concentration of neutral sterols: –	Hylla et al, 1998
24 healthy subjects in a controlled, randomized, cross-over study - 2 weeks	- RS2 (≈20g/d) - RS3 (≈25g/d) - Low fibre diet	Feacal output : ++ Butyrate: ++	Jenkins et al, 1998
57 babies (5-12 months) with persistent diarrhoea	- Rice-based diet containing green banana (250g/L diet) - Rice-based diet	Intestinal permeability: ++ Diarrhoea: –	Rabbani et al, 2001
23 patients with recently removed colonic adenomas in a randomized, controlled, parallel study - 4 weeks	- 45g amylomaize (28g/d RS2) in capsules - 45g maltodextrin in capsules	Cell proliferation: no effect Feacal output: no effect Faecal pH: no effect Feacal SCFA: no effect Faecal bile acids excretion: – Faecal secondary bile acids: –	Grubben et al, 2001

12 healthy volunteers in a controlled, cross-over study - 4 weeks	- High RS diet: Hylon VII in foods (RS: ≈ 55g/d) - Low RS (cornstarch, RS:2.8-3.7g/d)	Cell proliferation: no effect DNA adducts: ++	Wacker et al, 2002
20 volunteers with a family history of colorectal cancer in a randomized, cross-over study - 3 weeks	- Basal diet + RS (22g/d) + wheat bran (12g/d) - Basal diet + Wheat bran (12g/d) - Control diet	Feacal output: ++ Transit rate: ++ Faecal pH: –, only for RS Faecal SCFA: ++, only for RS Feacal phenols and ammonia: ++	Muir et al, 2004
8 healthy volunteers per fibre tested	- Phase 1: RS3 (10g/d) - Phase 2: RS3 (2.5, 5, 7.5 and 10g/d)	Prebiotic effects: ++ in phase 1 Prebiotic effects: ++ at 5 and 7.5g/d only in phase 2	Bouhnik et al, 2006
12 healthy volunteers with 3-14 bowel movements/ week, in a cross-over, randomized, double-blind, controlled study	- 25g/d Promitor™ Resistant Starch - Low fiber diet (<2g/d)	Fecal output: ++ Tolerance: good	Maki et al, 2009 (submitted)

++: increased or improved, –: decreased

Table 9.2

Animal studies investigating the effects of RS intake on luminal and epithelial colorectal cancer biomarkers (*Cont'd* p. 276)

Animal model	Intervention	Parameters measured	Author
Wistar rats (azoxymethane)	Dietary carbohydrate replaced with sucrose, cornstarch or RS (RS_2, 67g/100g)	ACF: –	Thorup et al, 1995
Sprague Dawley rats (Dimethylhydrazine)	3 or 10g/100g cellulose RS_3 (hydrolyzed high amylose maize starch)	Faecal output: ++ SCFA and butyrate production: ++ Tumor incidence: no effect Tumor size and multiplicity: no effect	Sakamoto et al, 1996
Sprague Dawley rats (dimethylhydrazine)	- Low RS, low fibre diet - 14.4g/100g diet RS (RS_2)	ACF: ++ Cell proliferation: ++ Faecal output: ++ Tumor size and multiplicity: ++	Young et al, 1996
C57BL/6 min mice	- RS-free diet (2% cellulose, no RS) - RS_3 (high amylose cornstarch, 18g/100g)	Gut-associated lymphoid tissue: no effect Tumor incidence: no effect	Pierre et al, 1997
Cannulated piglets	- RS-free diet - RS_2 (uncooked high amylose cornstarch) diet, 17% - RS_3 (retrograded high amylose cornstrarch) diet, 17%	Fecal nitrogen excretion: –, for RS_3 only Urine nitrogen excretion: –, for RS_3 only	Heijnen et al, 1997

Sprague Dawley rats (dimethylhydrazine)	- Retrograded high-amylose cornstarch (RS_3), 25% - RS_3, 25% + vitamin A diet Control diet	ACF: – Faecal output: ++ Faecal pH: – Butyrate: ++	Cassand et al, 1997
Sprague Dawley rats (dimethylhydrazine)	- RS-free diet (2% cellulose) - 25g/100g RS_3 (retrograded amylose maize starch)	ACF: no effect Caecal pH: – Faecal weight and output: ++ β-glucuronidase: –	Mazière et al, 1998
Human-flora associated Fisher rats	- Retrograded amylose (15g/100g diet) - RS free diet	Ammonia: – β-glucuronidase: – Caecal SCFA: ++ Cell proliferation: ++ Prebiotic effect: ++	Silvi et al, 1999
Min mice	RS free diet 1:1 potato RS and high-amylose maize diet	Tumor incidence: ++	Williamson et al, 1999
BDIX rats	- Low-fiber control diet - RS_3 (30%)	ACF: – Caecal butyrate: ++ Mucosal cell proliferation: no effect	Perrin et al, 2001
Wistar rats (IQ)	- RS free diet - Potato starch and high amylose maize starch (35%)	Excretion of the food carcinogen, Faecal output: ++ IQ: ++ Transit : ++	Ferguson et al, 2003

Table 9.2

Animal model	Intervention	Parameters measured	Author
Sprague Dawley rats	15g/100g casein with or without ≈50% Hi-Maize or 25g/100g casein with or without ≈50% Hi-Maize	Caecal SCFA: ++ DNA damage: – p-cresol: – Thinning of mucosal layer: –	Toden et al, 2005
Sprague Dawley rats (azoxymethane)	Low RS diet + 1% lyophilized *Lactobacillus acidophilus* and *Bifidobacterium lactis* - 10% RS diet (Hi-Maize) +1% lyophilized probiotics	Acute apoptotic response: ++ Cecal pH: – Cell proliferation: ++ Crypt column height: ++ Fecal and caecal SCFA: ++ Prebiotic effect : ++	Le Leu et al, 2005
Rats	- RS-free diet - Retrograded potato debranched maltodextrin (RSA(RS3), 5.7g/100g - RSB(RS3), 5.7g/100g)	Bile acids fecal excretion: ++ Caecal pH: – Caecal SCFA: ++ Fecal output: ++, for RSA	Dongowski et al, 2005
Sprague Dawley rats (DMH)	- RS-free diet - Heat-treated Novelose 330 (RS3, 10%)	Apoptosis: ++ Epithelial proliferation: – Tumor incidence: – Mucin properties: ++ for acidic mucin	Bauer-Marinovic et al, 2006
Wistar rats	- Heat-treated Novelose 330 - Normal Novelose 330	Caecal and colonic pH: – Crypt length: ++ Formation of secondary bile acids: – Fecal bile acids: ++ SCFA, butyrate: ++ Prebiotic effect: ++	Jacobash et al, 2006

++: increased or improved, –: decreased

In most of the studies, RS intake (17 g/d up to 50 g/d) was shown to increase fecal output, to increase fecal SCFA and to decrease fecal pH (Birkett et al., 1996; Cummings et al., 1996; Heijnen et al., 1998; Hylla et al., 1998; Jenkins et al., 1998; Maki et al., 2009; Muir et al., 2004; Phillips et al., 1995; van Munster et al., 1994).

The effect of RS consumption on fecal output is dose-dependent and increasing doses are associated with increasing recoverable starch in stools (Phillips et al., 1995). An increased fecal output is generally seen as being beneficial to gut health as it helps dilute the carcinogenic compounds and reduce thus their time contact with the epithelium.

The reduction of the fecal pH reflects an acidification of the colonic lumen due to the production of SCFA, notably butyrate linked to the fermentation of RS by the microbiota. The fact that RS favours butyrate production has been largely demonstrated *in vitro* (Brouns et al., 2007; Fassler et al., 2006, 2007) and in animal studies (Cassand et al., 1997; Dongowski et al., 2005; Jacobasch et al., 2006; Le Leu et al., 2005; Perrin et al., 2001; Sakamoto et al., 1996; Silvi et al., 1999; Toden et al., 2005). Butyrate plays a critical role in mucosal physiology and metabolism, by providing 50% of the daily energy requirements of the colonic mucosa, while also being implicated in cellular differentiation and proliferation. It has been seen *in vitro* that butyrate could be involved in cancer prevention through hyperacetylation of histone proteins in the cell nucleus (Tran et al., 1998) and selectively induce apoptosis in colon cancer cells but not in healthy colonocytes (Ruemmele et al., 2003).

The fermentation of RS induces changes in the microbiota metabolism pattern reducing either the numbers and/or activities of putrefactive bacteria in the colon, leading to a reduction in enzyme activities involved in carcinogenic pathways (e.g., β-glucuronidase) as well as toxic metabolites such as amines, indoles, p-cresol (Birkett et al., 1996; Heijnen and Beynen, 1997; Hylla et al., 1998). The use of protein fermentation products as a source of nitrogen for the microbiota to grow favours a reduction of fecal ammonia and phenols (Birkett et al., 1996; Muir et al., 2004). As protein fermentation products seem likely to be carcinogenic, this might be an additional mechanism by which RS might be protective. A low colonic pH is also associated with a decreased conversion rate of primary to secondary bile acids, which are thought together with other toxic metabolites like ammonia, phenol, amines, etc, to impact fecal water cytoxicity. In this respect, some human studies have shown that RS intake, due to its fermentation in the colon, can help reduce the production of secondary

bile acids (Grubben et al., 2001; Hylla et al., 1998; van Munster et al., 1994). Seeing the fact that RS intake helps in reducing the amount of several toxic compounds in the colon lumen via several mechanisms, a reduction of the fecal water cytoxicity is expected and this has been observed in a human study (van Munster et al., 1994) as well as in *in vitro* studies (Fassler et al., 2007).

One study in humans did consistently not show any effect (Grubben et al., 2001). That study was conducted in subjects with recently removed colonic adenomas, and the RS was administered in capsule form. They also failed to observe any changes in fecal fermentation, which suggests that the capsules did not effectively release the RS.

Generally speaking, similar effects as in humans have been observed in animals for luminal biomarkers of gut health. That is to say that in all animal studies, RS intake has favored butyrate production, increased fecal output, decreased pH, enzyme activity, ammonia, p-cresol and other toxic metabolites (Dongowski et al., 2005; Ferguson et al., 2003; Jacobasch et al., 2006; Maziere et al., 1998; Perrin et al., 2001; Sakamoto et al., 1996; Silvi et al., 1999; Toden et al., 2005; Young et al., 1996).

In rats, challenged with 1,2-dimethylhydrazine or azoxymethane or again in genetically modified mice models (min mice), the effects of RS intake on colorectal neoplasia and on epithelial markers of colorectal cancer have also been investigated (*Table 9.2*). However, depending on the type of animal model, on the nature and the amount of carcinogenic compounds used to induce colorectal cancer in rats, on the feeding time period, the effects of RS intake on aberrant crypt foci (ACF), cell proliferation, DNA damages, tumor incidence are highly variable. It is therefore very difficult to draw any conclusions on RS intake on markers of colorectal-cancers in animal studies. However, whether these particular animal models are relevant to investigate nutritional benefits of RS on gut health is a question mark as they induce drastic effects that do not reflect what occurs in physiological conditions and may wipe out any potential bioactive-associated preventive effects.

Toden et al. (2005) in this respect used a more appropriate rat model, which consisted in feeding rats with a western type diet enriched in proteins. They demonstrated that RS fermentation in the colon is beneficial to health in the sense that it helps counteract the deleterious effect induced by a high protein intake. The high protein diet resulted in a twofold increase in damage to colonocyte DNA compared to a low-protein diet. This was associated with thinning of the colonic mucosa barrier and increased level of p-cresol. The addition of

RS (high amylose starch) to the diet increased SCFA and attenuated DNA damages, suggesting protection against genotoxic agent and lesser genotoxocity of the fecal water.

These observations have been corroborated by a recent study that investigated the effects of *in vitro* fermentation products of *in-vitro*-digested or *in-vivo*-digested RS2 and RS3 on Caco-2 cells (Fassler et al., 2007). Compared to control, the cytotoxicity, anti-genotoxicity against hydrogen peroxide (comet assay) and the effect on barrier function measured by trans-epithelial electrical resistance of fermented samples of RS were determined. Batch fermentation of RS resulted in an anti-genotoxic activity ranging from 9–30% decrease in DNA damage for all the samples. Additionally, *in vitro* batch fermentation of RS caused an improvement in integrity across the intestinal barrier by approximately 22% for all the samples.

9.3 Other Starch-Derived Fibers with Potential Prebiotic Effects

Many new starch-derived prebiotic candidates are now available (e.g., Nutriose®, Fibersol-2® and PROMITOR™ Soluble Gluco Fiber).

Made from starch, Nutriose® can be described as a resistant dextrin. During the process of dextrinisation, the starch undergoes a degree of hydrolysis followed by repolymerization. It is this repolymerization step that makes starch become indigestible, due to many α 1,6, α 1,2 and α 1,3 linkages. According to the AOAC method 2001–2003, Nutriose® contains 85% fiber (Lefranc-Millot, 2008). The calorific value of Nutriose® has been reported to be 1.7 kcal/g (Lefranc-Millot, 2008) and is claimed to be consistent with clinical determination in healthy young men (Vermorel et al., 2004) and to be in agreement with the consensual calorific value of soluble dietary fibers (Livesey, 1992). Only 15% is enzymatically digested in the small intestine, while 75% reaches the colon where it is slowly fermented and 10% is excreted in fecal matters (van den Heuvel et al., 2005).

Fibersol-2® is a spray-dried powder produced by a pyrolysis and a controlled enzymatic hydrolysis of cornstarch. It was estimated that most of Fibersol-2 escapes digestion in the upper gastrointestinal tract and that 90% reaches the colon, where half of this fraction is fermented by the microbiota (Flickinger et al., 2000).

9.3.1 Introduction to PROMITOR™ Soluble Gluco Fiber (SGF)

9.3.1.1 Regulatory Status

PROMITOR™ Soluble Gluco Fiber is a regular food ingredient in the EU and can be labeled as "Soluble Gluco Fiber," "Glucose Syrup" or "Dried Glucose Syrup." The name "Soluble Gluco Fiber" is consistent with EU Directive 2000/13/EU as amended on labeling, presentation and advertising of foodstuffs as it indicates both the precise nature of the food in that it is a glucose type of food and distinguishes the fiber content of the glucose syrup. SGF is a non-genetically modified source of dietary fiber, which enables the use of fiber nutrient content claims (contains fiber, source of fiber, high fiber) where relevant regulatory conditions are met.

In the US, PROMITOR™ Soluble Corn Fiber (SGF in EU) is GRAS and can be labeled as "Soluble Corn Fiber," "Corn Syrup," or "Corn Syrup Solids."

9.3.1.2 Dietary Fiber Content

SGF is obtained from a partially hydrolyzed starch-made glucose syrup, using an existing production process that yields approximately 70–85% fiber with exceptional color, clarity and flavor (*Figure 9.3*).

SGF has a typical analysis of approximately 72% total dietary fiber per AOAC method 2001.03. Highly water soluble fibers such as SGF and some resistant maltodextrins contain digestion-resistant material that is not precipitated by the addition of ethanol as prescribed in the 991.43 method.

In three different human trials SGF has demonstrated a consistent glycemic response, approximately 30% that of rapidly digestible carbohydrates (i.e., glucose, maltodextrin) (Kendall et al., 2009). This correlates well to the amount of digestible carbohydrate (~70% fiber, ~30% digestible carbohydrate) based on the AOAC 2001.03 method.

9.3.1.3 Calorific Value

SGF, has a calorific content of 2 kcal/g (Fastinger et al., 2007). True metabolizable energy content, was determined in an *in vivo* avian model which utilizes bomb calorimetry of the food prior to consumption by the animal and

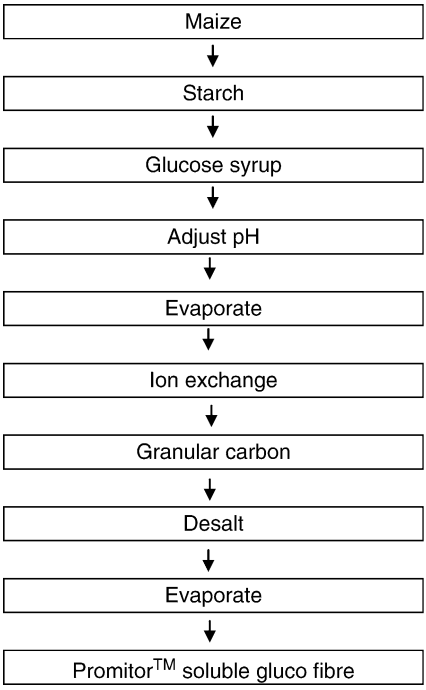


Figure 9.3
Production process of PROMITOR™ Soluble Gluco Fiber.

subsequent bomb calorimetry of the collected waste (urine and feces) after consumption. Additionally, glycemic response studies in humans support this calorific value (Kendall et al., 2007, 2008). Using the calculation (4 kcal/g × 30% digestible), SGF would have 1.2 kcal/g. However, energy yield from fermentation cannot be estimated by the blood glucose response and likely yields a small amount of additional kcals (<1 kcal). *In vitro* analysis has also shown it to be approximately 25% digestible, 50% fermented and 25% unchanged. Using a fermentation value of 2 kcal/g, as suggested by (Oku and Nakamura, 2002), the following calculations yield a calorific value for Soluble Gluco Fiber of 2 kcal/g.

$$\begin{aligned}\text{Soluble Gluco Fiber kcal/g} &= (4\ \text{kcal/g}) \times (25\%\ \text{digestible}) \\ &\quad + (2\ \text{kcal/g}) \times (50\%\ \text{fermentable}) \\ &\quad + (0\ \text{kcal/g}) \times (25\%\ \text{unchanged}) \\ &= 2\ \text{kcal/g}\end{aligned}$$

9.3.1.4 Digestive Tolerance

The gastrointestinal tolerance has been assessed in human trials and was shown to be well tolerated up to doses as high as 25 g/d. Stewart et al conducted a study in which subjects (n = 20, 10 men, 10 women) consumed 12 g fiber/d SGF for 14 consecutive days (Stewart et al., 2008, 2009). Subjects were asked to report gastrointestinal symptoms (cramping, bloating, stomach noise, flatulence) on a 10 point scale (1 = minimal symptoms, 10 = severe symptoms). SGF was shown to be well tolerated (Kendall et al., 2009; Sanders et al., 2008). In an acute dose-response study (Kendall et al., 2009), tolerance was assessed in 22 healthy volunteers consuming three different doses (5 g, 15 g, 25 g) in acute conditions after an overnight fast. Subjects were asked to report gastrointestinal symptoms (belching, bloating, flatulence, nausea and diarrhoea) on a 100 mm VAS scale at 0, 15, 30, 45, 60, 90 and 120 min after consumption. It was also shown to be well tolerated in these conditions up to 25 g.

9.3.1.5 Applications

SGF is an easy-to-formulate fiber which functions like glucose syrup in most systems. It can be used as a partial or total replacement for regular glucose syrup, other sweeteners, or low calorie bulking agents such as polyols, whilst maintaining texture and mouthfeel.

With 2 kcal/g, it also reduces calories when replacing sugar/glucose syrups (4 kcal/g). It has little to no sweetness and so can be used in combination with high intensity sweeteners to balance sweetness to the level of the standard product (in accordance with the EU legislation on sweeteners use in foodstuffs – Directive EU 94/35). As a result of its high solubility and stability in acidic conditions it is suitable for high solids low pH food matrices such as fruit filling. Its low impact on flavor and viscosity – similar to glucose syrup (*Figure 9.4*) – allows to reduce sugar and calories significantly, while adding fiber which does not affect the food product's organoleptic quality.

Main applications include cereals bars and breakfast cereals, cookies and biscuits, snacks, beverages, yogurts, ice creams, desserts, fruit fillings, sauces, confections and processed meats.

Beverages

SGF is a 100% water soluble, fiber source which does not cause any sedimentation or dramatically increase viscosity as some hydrocolloids do. These negative effects

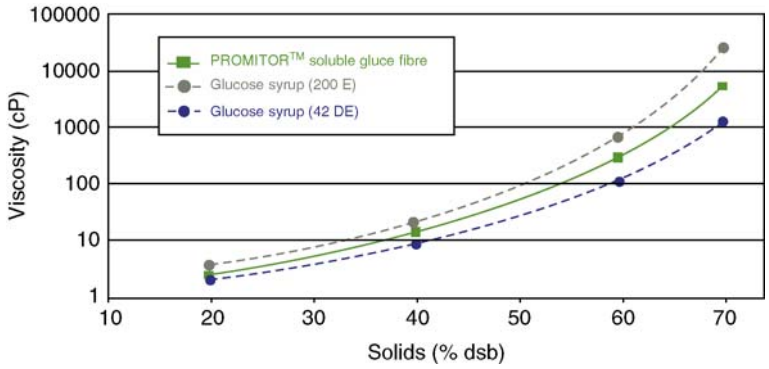


Figure 9.4
Viscosity of PROMITOR™ Soluble Gluco Fiber at 20°C.

can occur with other ingredients before or after heat treatment, dependent on the force and the type of shear applied. It also does not create cloudiness or turbidity. As a result, replacing sugar, either partially or totally, has no significant impact on the total "dry matter" or viscosity of the beverage, maintaining the original body and mouthfeel and avoiding the creation of a long or slimy structure.

As the energy is 2 kcal/g, it can help to deliver a significant sugar reduction. The sweetness is about 20% of the sweetness of sucrose, so adding a high intensity sweetener will create the desired taste. As a practical and simple example, replacing 4.5 g of sucrose per 100 mL in flavored and sweetened water with SGF will reduce energy load from 17.2 kcal/100 ml to less than 10 kcal/100 mL whilst adding 3 g fiber/100 mL. It can also be added to an existing beverage formulation and will then enhance its mouthfeel whilst simultaneously improving taste.

SGF is a maize starch derivative with a very bland flavor and does not contain any compounds responsible for off flavors sometimes caused during extraction and other processing stages. Its impact on the flavor of energy reduced drinks is not normally noticeable, with a smooth and non-grainy or powdery perception.

During beverage processing, pasteurization or flash pasteurization is often used to obtain microbiological stability. The combination of a heat treatment together with a low pH (as most beverages are) can damage some ingredients leading to breakdown before and during storage. SGF, however is stable throughout all normal processes in the beverage industry, which means there is no need to "overdose" it in formulations.

Bakery

Cereals bars are mainly composed of glucose syrups which work as a binder to maintain the cohesive structure of the particles of cereal and fruit. It is therefore particularly effective to replace glucose syrups with SGF, which can work as the sole binder thanks to its cohesive properties. Any reduction in sweetness can be compensated by ingredients such as fructose, although in many cases the dried part of the bar provides enough sweetness already. In addition SGF permits to achieve a fiber content of up to 35 g/100 g, corresponding to approximately 10 g/serving.

In moist matrices such as muffins, it decreases the water activity of the system and helps to maintain the stability of the product. In some cases a humectant (e.g., glycerol, sorbitol) can help to maintain the same water activity, ensuring that the product is preserved.

In biscuit-like and soft dough products, the low sweetness and the textural role of sugar, mean that the percentage of SGF incorporated does not generally exceed 50% of the total sugar.

Combining SGF and RS is a simple way to increase fiber content to reach the fiber amount necessary to make a "high fiber" claim, without affecting sensory characteristics of biscuits or muffin-like cakes whose regular flour based equivalents in the market have less than 1.5 g fiber/100 g.

Dairy

Because SGF behaves in a similar way to glucose syrup, it can effectively replace sugar while adding fiber in dairy products. At the same time it can contribute to a richer texture and a similar mouthfeel in low fat or non fat dairy products compared to full fat references.

Dairy processing typically includes heat treatment and homogenization steps which are synonymous to high shear, high temperature conditions. The stability in these harsh conditions makes it ideal for such dairy products.

Completely soluble at acidic and neutral pH, SGF adds texture and fibers with a smooth non sandy or powdery mouthfeel. For instance, it will compensate for the lack of body of a low fat and/or low sugar dairy dessert mousse, while increasing fiber and enhancing sensory characteristics.

It is also suitable for formulating fermented products due to its survivability during fermentation and can be added at the beginning of the process with no loss of fibers. (*Figure 9.5*).

In fruit preparations, it can replace the sweetener (either partly or totally) without impacting texture, while maintaining the pleasant mouthfeel stemming from its glucose syrup-like viscosity.

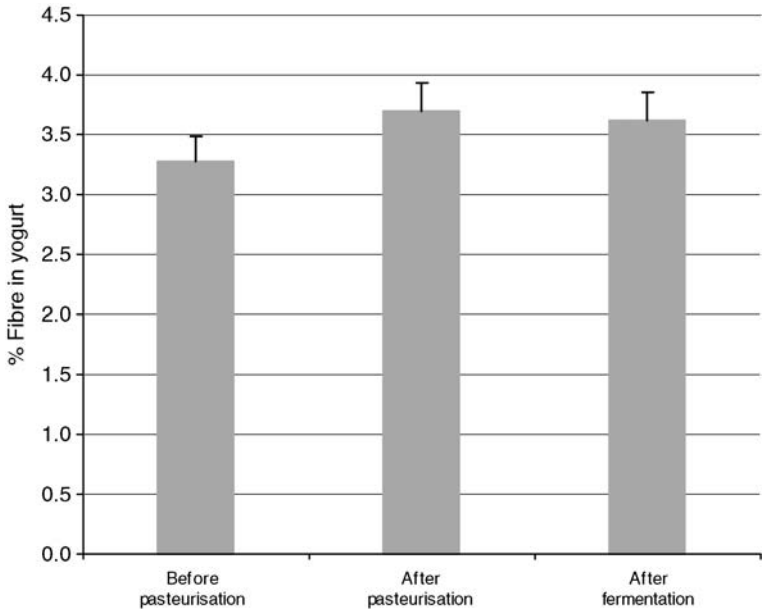


Figure 9.5
Stability of PROMITOR™ Soluble Gluco Fiber under Yogurt processing.

As SGF demonstrates high stability at low pH (pH < 4), it is particularly suitable for fruit preparations – where typical pH is around 3.8 to ensure good microbial stability during shelf-life – offering shelf-life stability with no loss of fiber content. Any sweetness loss is usually compensated by a high intensity sweetener such as sucralose.

In desserts and ice creams, SGF will add texture while replacing sweeteners (sugar or glucose syrup) and/or some fat, and will improve the nutritional profile of the end product. For instance, it is possible to achieve 30% fat and 60% sugar reductions in ice creams. In such formulations, it will help to keep a creamy taste and a mouthfeel similar to the full fat and sugar alternative.

9.3.2 Growing and Preliminary Evidence on Prebiotic Properties of New Starch-Derived Fibers

Fifteen grams per day Nutriose® intake over 2 weeks has been reported to decrease fecal pH and to reduce significantly *Clostridium perfringens* in humans (Lefranc-Millot, 2008).

Fibersol-2® has been shown to increase both bowel regularity, fecal volume and to favor growth of bifidobacteria in 20 healthy volunteers with a fecal frequency lower than three times a week (Satouchi et al., 1993). In this study, it has been observed that consumption of 3.75 g/d of Fibersol-2® for 5 days increased weekly fecal frequency from 2.6 times to 4.0 times and doubled fecal output. Bifidobacteria counts were also significantly increased. The prebiotic properties of Fibersol-2® have also been demonstrated in dogs (Flickinger et al., 2000). Though, a recent study, during which 38 healthy volunteers consumed 7.5 and 15 g Fibersol-2® over 3 weeks showed that resistant maltodextrin supplementation altered ($p < 0.05$) bacterial populations from baseline to treatment and increased butyrate production, but failed showing a significant effect ($p = 0.12$) on fecal *Bifidobacterium* populations during the treatment period (Fastinger et al., 2008).

SGF has been shown to be a potential prebiotic in *in vitro* studies at TNO (Maathuis et al., 2008). As described previously, the aim of the study was to investigate the effect of five newly developed maize-based fibers on the activity and composition of the microbiota in the colon. The fibers were pre-digested, mono- and di-saccharides were removed, and the residual polymer was used to assess the production of microbial metabolites and changes in composition of the microbiota using a dynamic, validated, *in vitro* model of the large intestine (TIM-2). Microbial metabolites analysis showed an increase in health-promoting metabolites (short-chain fatty acids) and a reduction in toxic metabolites from protein fermentation compared to the poorly-fermentable control (cellulose). Using microarray technology to compare multiple species and groups of colonic microbiota, it was found to promote the growth of beneficial bacteria such as bifidobacteria (*Figure 9.2*).

Human studies are ongoing to confirm the prebiotic properties of SGF.

9.4 Conclusion

The prebiotic effects of RS are promising. Existing data suggest that RS would be able to positively impact immune response, modulate inflammation, improve mineral absorption and help maintain a good colic function. Preliminary data on PROMITOR™ Resistant Starch suggest similar properties as those that have been reported so far for RS2 and RS3.

New soluble non-viscous fibers like PROMITOR™ Soluble Gluco Fiber are easy to integrate into new or existing formulations without compromising flavor or texture. Very well tolerated and clean tasting, the new generation of

fibers can help develop new health-plus versions of products in a wide range of food categories. In particular their low calorific value (0–2 kcal/g), makes them preferable alternatives to high calorific ingredient (sugar 4 kcal/g, fat 9 kcal/g). The fact that they can compensate for a lack of body and texture in many low calorie – sugar or fat reduced – products means that they are frequently used in the dairy and bakery sectors in particular. They have also shown high process and acid stability allowing manufacturers to formulate and guarantee fiber content (and other associated benefits) throughout the entire shelf life of products.

References

Asp NG (1997) Resistant starch – an update on its physiological effects. Adv Exp Med Biol 427:201–210

Bauer-Marinovic M, Florian S, Müller-Schmehl K, Glatt H, Jacobasch G (2006) Dietary resistant starch type 3 prevents tumour induction by 1,2-dimethylhydrazine and alters proliferation, apoptosis and dedifferentiation in rat colon. Carcinogenesis 27:1849–1859

Birkett A, Muir J, Phillips J, Jones G, O'Dea K (1996) Resistant starch lowers fecal concentrations of ammonia and phenols in humans. Am J Clin Nutr 63:766–772

Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, Flourie B, Brouns F, Bornet FR (2004) The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. Am J Clin Nutr 80:1658–1664

Brouns F, Arrigoni E, Langkilde AM, Verkooijen I, Fassler C, Andersson H, Kettlitz B, van Nieuwenhoven M, Philipsson H, Amado R (2007) Physiological and metabolic properties of a digestion-resistant maltodextrin, classified as type 3 retrograded resistant starch. J Agric Food Chem 55:1574–1581

Brown I, Warhurst M, Arcot J, Playne M, Illman RJ, Topping DL (1997) Fecal numbers of bifidobacteria are higher in pigs fed Bifidobacterium longum with a high amylose cornstarch than with a low amylose cornstarch. J Nutr 127:1822–1827

Burkitt DP, Trowell HC (1975) Refined carbohydrate foods and disease: some implications of dietary fibre. Academic Press, London, p. 369

Burns AJ, Rowland IR (2000) Anticarcinogenicity of probiotics and prebiotics. Curr Issues Intest Microbiol 1:13–24

Buttriss JL, Stokes CS (2008) Dietary fibre and health: an overview. Nutr Bull 33:186–200

Cassand P, Maziere S, Champ M, Meflah K, Bornet F, Narbonne JF (1997) Effects of resistant starch- and vitamin A-supplemented diets on the promotion of precursor lesions of colon cancer in rats. Nutr Cancer 27:53–59

Cassidy A, Bingham SA, Cummings JH (1994) Starch intake and colorectal cancer risk: an international comparison. Br J Cancer 69:937–942

Crittenden RG, Morris LF, Harvey ML, Tran LT, Mitchell HL, Playne MJ (2001) Selection of a Bifidobacterium strain to complement resistant starch in a synbiotic yoghurt. J Appl Microbiol 90:268–278

Cummings JH, Beatty ER, Kingman SM, Bingham SA, Englyst HN (1996)

Digestion and physiological properties of resistant starch in the human large bowel. Br J Nutr 75:733–747

Dongowski G, Jacobasch G, Schmiedl D (2005) Structural stability and prebiotic properties of resistant starch type 3 increase bile acid turnover and lower secondary bile acid formation. J Agric Food Chem 53:9257–9267

Fassler C, Arrigoni E, Venema K, Brouns F, Amado R (2006) In vitro fermentability of differently digested resistant starch preparations. Mol Nutr Food Res 50:1220–1228

Fassler C, Gill CI, Arrigoni E, Rowland I, Amado R (2007) Fermentation of resistant starches: influence of in vitro models on colon carcinogenesis. Nutr Cancer 58:85–92

Fastinger N, Knapp B, Guevara M, Parsons C, Swanson K, Fahey G (2007) Glycemic response and metabolizable energy content of novel maize-based soluble fibers F4–809, F4–810 and F4–810LS using canine and avian models. FASEB J 21:A744

Fastinger ND, Karr-Lilienthal LK, Spears JK, Swanson KS, Zinn KE, Nava GM, Ohkuma K, Kanahori S, Gordon DT, Fahey GC Jr (2008) A novel resistant maltodextrin alters gastrointestinal tolerance factors, fecal characteristics, and fecal microbiota in healthy adult humans. J Am Coll Nutr 27:356–366

Ferguson LR, Zhu S, Kestell P (2003) Contrasting effects of non-starch polysaccharide and resistant starch-based diets on the disposition and excretion of the food carcinogen, 2-amino-3-methylimidazo[4,5-f] quinoline (IQ), in a rat model. Food Chem Toxicol 41:785–792

Flickinger EA, Wolf BW, Garleb KA, Chow J, Leyer GJ, Johns PW, Fahey GC Jr (2000) Glucose-based oligosaccharides exhibit different in vitro fermentation patterns and affect in vivo apparent nutrient digestibility and microbial populations in dogs. J Nutr 130:1267–1273

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412

Gray J (2006) Dietary fibre: definition, analysis, physiology & health. ILSI Europe Consise monograph series, pp. 1–44

Grubben MJ, van den Braak CC, Essenberg M, Olthof M, Tangerman A, Katan MB, Nagengast FM (2001) Effect of resistant starch on potential biomarkers for colonic cancer risk in patients with colonic adenomas: a controlled trial. Dig Dis Sci 46:750–756

Hampson DJ, Robertson ID, La T, Oxberry SL, Pethick DW (2000) Influences of diet and vaccination on colonisation of pigs by the intestinal spirochaete Brachyspira (Serpulina) pilosicoli. Vet Microbiol 73:75–84

Heijnen ML, Beynen AC (1997) Consumption of retrograded (RS3) but not uncooked (RS2) resistant starch shifts nitrogen excretion from urine to feces in cannulated piglets. J Nutr 127:1828–1832

Heijnen ML, van Amelsvoort JM, Deurenberg P, Beynen AC (1998) Limited effect of consumption of uncooked (RS2) or retrograded (RS3) resistant starch on putative risk factors for colon cancer in healthy men. Am J Clin Nutr 67:322–331

Hylla S, Gostner A, Dusel G, Anger H, Bartram HP, Christl SU, Kasper H, Scheppach W (1998) Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention. Am J Clin Nutr 67:136–142

Institute of Medicine, Food and Nutrition Board (2006) Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Fatty acids, Cholesterol, Protein and Amino acids. National Academy of Sciences

Jacobasch G, Dongowski G, Schmiedl D, Muller-Schmehl K (2006) Hydrothermal treatment of Novelose 330 results in high yield of resistant starch type 3 with beneficial prebiotic properties and decreased secondary bile acid formation in rats. Br J Nutr 95:1063–1074

Jacobasch G, Dongowski G, Schmiedl D, Muller-Schmehl K (2006) Hydrothermal treatment of Novelose 330 results in high yield of resistant starch type 3 with beneficial prebiotic properties and decreased secondary bile acid formation in rats. Br J Nutr 95:1063–1074

Jenkins DJ, Vuksan V, Kendall CW, Wursch P, Jeffcoat R, Waring S, Mehling CC, Vidgen E, Augustin LS, Wong E (1998) Physiological effects of resistant starches on fecal bulk, short chain fatty acids, blood lipids and glycemic index. J Am Coll Nutr 17:609–616

Kendall C, Esfahani A, Hoffman A, Evans A, Sanders LJ,AR, Vidgen E, Potter S (2008) Effect of novel maize-based dietary fibers on postprandial glycemia and insulinemia. J Am Coll Nutr 27:711–718

Kendall C, Esfahani A, Sanders L, Potter S, Jenkins D (2009) Resistant starch reduces postprandial glycemic and insulinemic response and increases satiety in humans. FASEB J, in press

Kendall C, Josse A, Potter S, Hoffman A, Jenkins D (2007) Effect of novel maize-based dietary fibers on postprandial glycemia. FASEB J 21:A177

Knapp BK, Parsons CM, Swanson KS, Fahey GC Jr (2008) Physiological responses to novel carbohydrates as assessed using canine and avian models. J Agric Food Chem 56:7999–8006

Le Blay GM, Michel CD, Blottiere HM, Cherbut CJ (2003) Raw potato starch and short-chain fructo-oligosaccharides affect the composition and metabolic activity of rat intestinal microbiota differently depending on the caecocolonic segment involved. J Appl Microbiol 94:312–320

Le Leu RK, Brown IL, Hu Y, Bird AR, Jackson M, Esterman A, Young GP (2005) A synbiotic combination of resistant starch and Bifidobacterium lactis facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. J Nutr 135:996–1001

Lefranc-Millot C (2008) NUTRIOSE® 06: a useful soluble dietary fibre for added nutritional value. Nutr Bull 33:234–239

Lenoir-Wijnkoop I, Sanders ME, Cabana MD, Caglar E, Corthier G, Rayes N, Sherman PM, Timmerman HM, Vaneechoutte M, Van Loo J, Wolvers DA (2007) Probiotic and prebiotic influence beyond the intestinal tract. Nutr Rev 65:469–489

Lesmes U, Beards EJ, Gibson GR, Tuohy KM, Shimoni E (2008) Effects of resistant starch type III polymorphs on human colon microbiota and short chain fatty acids in human gut models. J Agric Food Chem 56:5415–5421

Livesey G (1992) The energy values of dietary fibre and sugar alcohols for man. Nutr Res Rev 5:61–84

Lopez HW, Levrat-Verny MA, Coudray C, Besson C, Krespine V, Messager A, Demigne C, Remesy C (2001) Class 2 resistant starches lower plasma and liver lipids and improve mineral retention in rats. J Nutr 131:1283–1289

Maathuis A, Hoffman A, Evans A, Sanders L, Venema K (2008) Digestibility and prebiotic potential of nondigestible carbohydrate fractions from novel maize-based fibers in a dynamic in vitro model of the human intestine. FASEB J 22:1089–1087

Macfarlane S (2008) Microbial biofilm communities in the gastrointestinal tract. Journal of clinical gastroenterology 42(Suppl. 3) Pt 1:S142–S143

Maki K, Sanders L, Reeves M, Kaden V, Cartwright Y (2009) Effects of resistant starch vs wheat bran on laxation in healthy adults. FASEB J, in press

Marsono Y, Illman RJ, Clarke JM, Trimble RP, Topping DL (1993) Plasma lipids and large bowel volatile fatty acids in pigs fed on white rice, brown rice and rice bran. Br J Nutr 70:503–513

Martin B, Lachcik P, Story J, Weaver C (2009) Calcium absorption, retention and bone density are enhanced by different fibers in male Sprague Dawley rats. FASEB J, in press

Maziere S, Meflah K, Tavan E, Champ M, Narbonne JF, Cassand P (1998) Effect of resistant starch and/or fat-soluble vitamins A and E on the initiation stage of aberrant crypts in rat colon. Nutr Cancer 31:168–177

Muir JG, Yeow EG, Keogh J, Pizzey C, Bird AR, Sharpe K, O'Dea K, Macrae FA (2004) Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. Am J Clin Nutr 79:1020–1028

Oku T, Nakamura S (2002) Digestion, absorption, fermentation, and metabolism of functional sugar substitutes and their available energy. Pure Appl Chem 74:1253–1261

Perrin P, Pierre F, Patry Y, Champ M, Berreur M, Pradal G, Bornet F, Meflah K, Menanteau J (2001) Only fibres promoting a stable butyrate producing colonic ecosystem decrease the rate of aberrant crypt foci in rats. Gut 48:53–61

Phillips J, Muir JG, Birkett A, Lu ZX, Jones GP, O'Dea K, Young GP (1995) Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. Am J Clin Nutr 62:121–130

Rabbani GH, Teka T, Zaman B, Majid N, Khatun M, Fuchs GJ (2001) Clinical studies in persistent diarrhoea: dietary management with green banana or pectin in Bangladeshi children. Gastroenterology 121:554–560

Ramakrishna BS, Subramanian V, Mohan V, Sebastian BK, Young GP, Farthing MJ, Binder HJ (2008) A randomized controlled trial of glucose versus amylase resistant starch hypo-osmolar oral rehydration solution for adult acute dehydrating diarrhoea. PLoS ONE 3:e1587

Ramakrishna BS, Venkataraman S, Srinivasan P, Dash P, Young GP, Binder HJ (2000) Amylase-resistant starch plus oral rehydration solution for cholera. N Engl J Med 342:308–313

Roberfroid M (2007) Prebiotics: the concept revisited. J Nutr 137:830S–837S

Ruemmele FM, Schwartz S, Seidman EG, Dionne S, Levy E, Lentze MJ (2003) Butyrate induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway. Gut 52:94–100

Sakamoto J, Nakaji S, Sugawara K, Iwane S, Munakata A (1996) Comparison of resistant starch with cellulose diet on 1,2-dimethylhydrazine-induced colonic carcinogenesis in rats. Gastroenterology 110:116–120

Sanders L, Kendall C, Maki K, Stewart M, Slavin J, Potter S (2008) A novel maize-based dietary fiber is well tolerated in humans. FASEB J 22:lb761

Satouchi M, Wakabayashi S, Ohkuma K, Fuyuwara K, Matsouka A (1993) Effects of indigestible dextrine on bowelmovements. Jpn J Nutr 51:31–37

Schley PD, Field CJ (2002) The immune-enhancing effects of dietary fibres and prebiotics. Br J Nutr 87(Suppl. 2): S221–S230

Scholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Acil Y, Gluer CC, Schrezenmeir J (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J Nutr 137:838S–846S

Silvi S, Rumney CJ, Cresci A, Rowland IR (1999) Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with faeces from Italian and UK donors. J Appl Microbiol 86:521–530

Stewart M, Nikhanj S, Timm D, Thomas W, Slavin J (2009) Four different fibers from maize and tapioca are well tolerated in a placebo-controlled study in humans. FASEB J, in press

Toden S, Bird AR, Topping DL, Conlon MA (2005) Resistant starch attenuates colonic DNA damage induced by higher dietary protein in rats. Nutr Cancer 51:45–51

Topping DL, Fukushima M, Bird AR (2003) Resistant starch as a prebiotic and synbiotic: state of the art. Proc Nutr Soc 62:171–176

Tran CP, Familari M, Parker LM, Whitehead RH, Giraud AS (1998) Short-chain fatty acids inhibit intestinal trefoil factor gene expression in colon cancer cells. Am J Physiol 275:G85–G94

Tuohy KM, Rouzaud GC, Bruck WM, Gibson GR (2005) Modulation of the human gut microflora towards improved health using prebiotics – assessment of efficacy. Curr Pharm Des 11:75–90

van den Heuvel EG, Wils D, Pasman WJ, Saniez MH, Kardinaal AF (2005) Dietary supplementation of different doses of NUTRIOSE FB, a fermentable dextrin, alters the activity of faecal enzymes in healthy men. Eur J Nutr 44:445–451

van Munster IP, Tangerman A, Nagengast FM (1994) Effect of resistant starch on colonic fermentation, bile acid metabolism, and mucosal proliferation. Dig Dis Sci 39:834–842

Vermorel M, Coudray C, Wils D, Sinaud S, Tressol JC, Montaurier C, Vernet J, Brandolini M, Bouteloup-Demange C, Rayssiguier Y (2004) Energy value of a low-digestible carbohydrate, NUTRIOSE FB, and its impact on magnesium, calcium and zinc apparent absorption and retention in healthy young men. Eur J Nutr 43:344–352

Vos AP, M'Rabet L, Stahl B, Boehm G, Garssen J (2007) Immune-modulatory effects and potential working mechanisms of orally applied nondigestible carbohydrates. Crit Rev Immunol 27:97–140

Wang X, Brown IL, Khaled D, Mahoney MC, Evans AJ, Conway PL (2002) Manipulation of colonic bacteria and volatile fatty acid production by dietary high amylose maize (amylomaize) starch granules. J Appl Microbiol 93:390–397

Williamson SL, Kartheuser A, Coaker J, Kooshkghazi MD, Fodde R, Burn J, Mathers JC (1999) Intestinal tumorigenesis in the Apc1638N mouse treated with aspirin and resistant starch for up to 5 months. Carcinogenesis 20:805–810

Younes H, Coudray C, Bellanger J, Demigne C, Rayssiguier Y, Remesy C (2001) Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. Br J Nutr 86:479–485

Young GP, McIntyre A, Albert V, Folino M, Muir JG, Gibson PR (1996) Wheat bran suppresses potato starch–potentiated colorectal tumorigenesis at the aberrant crypt stage in a rat model. Gastroenterology 110:508–514