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Create Exceptional™

Fibersym® RW
RESISTANT WHEAT STARCH

TECHNICAL SPECIFICATIONS

NON GMO Project VERIFIED
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MGP®
Create Exceptional™

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ABOUT MGP

Founded in 1941 in Atchison, Kansas, under the original name of Midwest Solvents Company, but now MGP Ingredients Inc., the company has been serving the food and alcohol industry for nearly eight decades. Today, MGP is a leading producer and supplier of premium distilled spirits and specialty wheat proteins and starch food ingredients. It operates in two segments, Distillery Products and Ingredient Solutions.

The Ingredient Solutions segment provides specialty wheat proteins and starch food ingredients for use in a diverse range of food products. Through the years, MGP has pioneered the development of these specialty food ingredients, resulting in a wide range of superior ingredient solutions for a host of food product applications. These solutions provide a multitude of functions and benefits for bakery and prepared foods, including processing improvements, moisture management, shelf-life extension, fiber fortification, calorie reduction, fat reduction and texture enhancement. The company sells its products directly or through distributors to manufacturers and processors of finished packaged goods or to bakeries around the world.

MGP's applications scientists work closely with our sales team to provide technical support to customers by addressing specific needs and/or helping define the value and performance of our ingredients for targeted uses. They are available to work directly with customers at their facilities or at our fully equipped, state-of-the-art Technical Innovation Center to assist in the development of product formulations. Meanwhile, our research scientists direct their expertise and resources toward the development and refinement of new and innovative ingredients for the future.

In short, we recognize that our customers want a reliable partner who can effectively support their objectives and boost product success. With our specialty ingredient solutions, decades of experience and technical know-how, we at MGP are equipped to meet customers' needs.

ABOUT FIBERSYM® RW

Fibersym® RW, a phosphorylated cross-linked RS4 wheat starch, is an FDA-approved source of dietary fiber and is ideal for incorporation in foods that provide benefits related to health and wellness. It complies with FDA's new nutrition facts labeling regulations issued on May 27, 2016. Fibersym® is classified as RS4-type resistant starch, meaning it is indigestible in the upper gastrointestinal tract. It is a convenient and rich source of dietary fiber that can be formulated in a wide array of foods with minimal processing adjustments. Possessing a clean flavor, smooth texture and white appearance, in combination with its low water-holding properties, Fibersym® allows formulators to boost the fiber content and lower calories of a diverse line of products while delivering health benefits to consumers. Applications include bread, pizza crust, flour tortillas, pasta and noodles, cookies, English muffins, breakfast cereals, pastries and bakery mixes. Health-wise, this superb ingredient lowers postprandial blood glucose and insulin levels, lowers blood cholesterol, and reduces waist circumference and body fat percentage (which can reduce the risk of being overweight or obese). It promotes gastrointestinal health by increasing colonic fermentation/short-chain fatty acid production and contributing to positive modulation of colonic microflora.

Fibersym® contains a maximum of 0.4% phosphorus and a minimum of 90% total dietary fiber (dry basis) when analyzed according to AOAC Method 991.43. Fibersym's calorie count is 35.1 kcal/100 g or ~0.4 kcal/g using the calculation procedure in the new nutrition-facts labeling regulations.

Fibersym® is labeled "modified wheat starch" in the ingredient statement of food product packages. This label declaration complies with Title 21 of the Code of Federal Regulations Part 172.892 and the Food Allergen Labeling and Consumer Protection Act of 2004. Fibersym® is certified Non-GMO Project Verified and has received both Kosher and Halal certifications.

Key Points of Fibersym® RW:

- FDA-approved source of dietary fiber
- Fiber fortification (good/excellent source of fiber claim)
- Calorie reduction (~0.4 calories per gram)
- Beneficial physiological effects in humans
- 1:1 Flour replacement
- Low water holding capacity
- Smooth, non-gritty texture
- White, "invisible" fiber source
- Process tolerant
- Enhances crispiness

THE CONCEPT OF RESISTANT STARCH

During consumption of a food containing starch, approximately 5% digestion of starch occurs in the mouth during mastication through the action of salivary alpha-amylase. When the food reaches the stomach, it gets mashed by the churning action of this organ and pepsin starts digesting the protein component of the food. The high acidity in the stomach deactivates the salivary alpha-amylase. Thus, no enzymatic starch digestion happens in the stomach. The mashed food travels next to the small intestine where pancreatic enzymes and bile act on the food. Final digestion occurs in the brush border of the small intestinal epithelium, resulting in absorption of glucose, amino acids and fatty acids into the blood stream. The presence of glucose in the blood triggers the pancreas to produce the hormone insulin, which helps transport glucose into the different cells of the body to serve as a source of energy. Remnants of undigested foods (mainly dietary fiber) flow into the large intestine where they provide bulk or undergo microbial fermentation with the release of gases and short-chain fatty acids.

In 1982, while working on a testing methodology to quantify the content of non-starch polysaccharides (dietary fiber components) of foods by enzyme digestion, British scientists discovered that the "fiber residue" of bread and cooked and cooled potatoes contains enzyme-resistant starch. This was surprising since starch was widely thought to be a completely digestible carbohydrate as noted above. Those British investigators also found that raw starch from potato and green banana largely resisted digestion by mammalian alpha-amylase. This resistance to digestion was confirmed in humans when researchers recovered undigested starch in the effluents of ileostomy patients. (Note: An ileostomy is a surgical procedure in which the ileum of the small intestine is attached to the abdominal wall in order to bypass the large intestine. Digestive waste then exits the body through an artificial opening.) With the discovery that a low level of dietary starch is resistant to hydrolysis by digestive enzymes, the words "resistant starch" were coined.

A decade after resistant starch became firmly ingrained in the vernacular of food, nutrition and health professionals, the official definition for resistant starch was developed, which states that it is "the sum of starch and products of starch degradation not absorbed in the small intestines of healthy individuals". Resistant starch is included in the definition of dietary fiber, and is considered a third kind of fiber in addition to insoluble fiber and soluble fiber.

Not all resistant starches are the same. In fact, they are classified into five types. Type 1 or RS1 occurs in whole grains or in incompletely- or coarsely-milled cereal grains, seeds and legumes. Examples are cracked wheat, farina, semolina, red beans, pinto beans and white beans. The starch granules are encapsulated within a plant cell wall such that the digestive enzymes are prevented or delayed from having access to them. The gastrointestinal tract of humans lacks enzymes capable of degrading the components of plant cell walls in order to expose the physically-shielded starch granules. The amount of RS1 is affected by processing and can actually be decreased or eliminated by complete disintegration or fine milling. Flours and meals from high-amylose corn and flours from high-amylose barley and high-amylose wheat are commercially available for use as resistant starch sources in food product development.

Examples of type 2 resistant starch, or RS2, are starch granules from raw potato, unripe or green bananas, and high-amylose corn. These starch granules are resistant to enzymatic digestion by virtue of their inherent crystalline structure. Like RS1, the amount of RS2 can be affected by processing. Raw potato and raw banana starches completely lose resistance to enzyme digestion upon cooking. On the other hand, heat-moisture treatment of high-amylose starches enhances their level of RS2. High-amylose corn starches with varying levels of RS2 resistant starch are currently sold in the market.

Highly associated, crystalline amylose represents resistant starch belonging to type 3 or RS3. Highly associated or crystalline amylose forms during a process termed retrogradation, which occurs when an amylose-containing starch is cooked and cooled. The level of RS3 generated during retrogradation depends on starch concentration, and on time and temperature. In the commercial production of an RS3 ingredient, an aqueous slurry of starch is repeatedly heated and retrograded to generate an increasing level of RS3. The intimate association of amylose molecules of long chain-length results in the formation of a water-insoluble, partially crystalline structure which resists digestion. This type of resistant starch can be generated in processed foods as in cooked and cooled potato, and cooked and cooled rice, as well as in bread and corn flakes. RS3 products manufactured from tapioca starch, normal corn starch, and high-amylose corn starch are commercially available.

Type 4 resistant starch, or RS4, occurs in chemically modified starch. All forms of chemically modified starch have varying degrees of resistance to enzyme digestion. Some of MGP's modified wheat starch products contribute some resistance to digestion, but the magnitude of resistance is low and they offer no commercial value as a significant source of resistant starch. One form of modification as described in U.S. Patent 5,855,946 is accomplished by cross-linking starch with sodium trimetaphosphate in the presence of small amounts of sodium tripolyphosphate. Two products made by such a process are MGP's Fibersym® RW and FiberRite® RW. The cross-links in those two resistant starches are diesters of a phosphate molecule. Phosphate diester linkages are ubiquitous in nature, occurring in DNA, RNA, and phospholipids, to name a few. Also, the level of phosphate substitution in Fibersym® or FiberRite® is low; only ~3% of glucose repeat units are phosphorylated. Even so, those phosphate cross-links in Fibersym® and FiberRite® limit the swelling of their granules in water, and such a tight structure and possible steric inhibition at an amylase's active site inhibit enzymatic digestion. Other phosphorylated cross-linked RS4 products are commercially available such as those made from tapioca and potato starches.

The fifth type of resistant starch, or RS5, was recently introduced as a new type of resistant starch. A starch-lipid or amylose-lipid complex forms when starchy foods, or purified amylose-containing starches, are heated or cooked in the presence of a lipid molecule containing a single fatty-acid chain. This durable single-helical complex offers resistance to enzymatic digestion based on its insolubility and steric hindrance to the active site of an amylase. Currently, there is no commercially-manufactured RS5 resistant starch available in the market.

NEW FDA DEFINITION OF DIETARY FIBER

On May 27, 2016, The U.S. Food and Drug Administration (FDA) published a final rule in the Federal Register: "Food Labeling: Revision of the Nutrition and Supplement Facts Labels" (Federal Register, Vol. 81, No. 103, pp. 33742-33999). In a pertinent part, FDA explicitly defined "dietary fiber" as follows:

Dietary fiber includes: (1) Non-digestible soluble and insoluble (with 3 or more monomeric units) carbohydrates and lignin that are intrinsic and intact in plants; (2) isolated or synthetic non-digestible carbohydrates (with 3 or more monomeric units) that FDA has granted be included in the definition of dietary fiber, in response to a citizen petition FDA received demonstrating that such carbohydrates have a physiological effect(s) that is beneficial to human health; or (3) isolated or synthetic non-digestible carbohydrates (with 3 or more monomeric units) that are the subject of an authorized health claim.

Resistant starch (like Fibersym® RW) is included in the above dietary fiber definition as stated in (2) isolated or synthetic non-digestible carbohydrates (with 3 or more monomeric units) that FDA has granted be included in the definition of dietary fiber, in response to a citizen petition FDA received demonstrating that such carbohydrates have a physiological effect(s) that is beneficial to human health.

A resistant starch that has been extracted and isolated from flaked corn cereal, such that it is no longer part of the food matrix (intrinsic) and no longer consists of relevant food components (intact), often with an increased concentration of non-digestible carbohydrates, would be considered an isolated non-digestible carbohydrate. The term "isolated" is used to describe isolated non-digestible carbohydrates that are isolated from plant sources such that they are no longer intrinsic or intact. Some of these isolated fibers can be further modified. The term "synthetic" is used to describe synthetic non-digestible carbohydrates that are not isolated from plant sources, but rather chemically synthesized. An isolated or synthetic non-digestible carbohydrate needs to provide at least one beneficial physiological effect.

There is no sole or universal method for determining the dietary fiber content of food products. If a product contains only non-digestible carbohydrates that meet the proposed definition of dietary fiber, using AOAC Method 2009.01, AOAC Method 2011.25, or an equivalent method of analysis as given in the "Official Methods of Analysis of the AOAC International 19th edition, 2012" would be sufficient to quantify the dietary fiber content of a food. Equivalent methods include AOAC Method 985.29 and AOAC Method 991.43 for high-molecular weight dietary fiber, and AOAC Method 2001.03 for high-molecular weight dietary fiber and low-molecular weight soluble dietary fiber.

FDA agrees that AOAC Method 2009.01 and AOAC Method 2011.25 do not measure all forms of RS4, as in the case of phosphorylated cross-linked RS4 wheat starch (like Fibersym). In this case, a more appropriate method (for example, AOAC Method 991.43, AOAC Method 985.29, AOAC Method 2001.03, etc.) can be identified that can measure all of the RS4.

The Daily Value for dietary fiber was increased in 2016 by FDA from 25 grams a day to 28 grams a day, which represents a 12% increase. A caloric content of 2 kcal/g is to be used for soluble dietary fiber, whereas insoluble dietary fiber is considered non-caloric (0 kcal/g). In calculating the caloric contribution of carbohydrates in a food, digestible (available) carbohydrate is calculated to contribute 4 kcal/g and soluble non-digestible carbohydrate contributes 2 kcal/g.

Based on a citizen petition submitted by MGP on October 28, 2016, FDA granted the request on March 26, 2019 to amend the definition of dietary fiber by adding Modified Wheat Starch, [Fibersym®RW and FiberRite®RW (cooked version of Fibersym)], to the existing list of isolated or synthetic non-digestible carbohydrates determined by the agency to have physiological effects that are beneficial to human health. Based on available evidence, FDA has determined that the scientific evidence suggests cross-linked phosphorylated RS4 like Fibersym® and FiberRite® can help reduce insulin levels following a meal containing a carbohydrate that raises blood glucose levels. This positive action by FDA recognizes the dietary fiber status of Fibersym® and FiberRite® under the new nutrition facts labeling regulations issued in 2016.

PROGRESS OF RS4 TECHNOLOGY

Fibersym® RW was launched commercially in 2003 based on an exclusive licensing agreement to MGP to practice the patented RS4 technology (U.S. Patent 5,855,946) from Kansas State University Research Foundation (Manhattan, KS). Since the phosphorylated cross-linked RS4 starch patent was issued in 1999, several technologies were developed and patents subsequently issued in support of the RS4 technology. In the U.S. Patent 8,753,705, biologically-active minerals like calcium, aluminum, copper, iron, magnesium, manganese, nickel, potassium, chromium, zinc and their mixtures were bound to phosphorylated cross-linked starch. The mineral-bound starch products are stable against heating in hot water followed by washing processes, but able to release the bound minerals after digestion. Cold water-swellable granular wheat starch was cross-linked with sodium trimetaphosphate, substituted with octenyl succinic anhydride and then complexed with aluminum sulfate (U.S. Patent 7,166,305). The product has excellent dry flow characteristics and ease of dispersability in hot or cold water. Interacted blends of phosphorylated cross-linked RS4 starch and gums/hydrocolloids (for example, sodium alginate, hydroxypropyl methyl cellulose, carboxymethyl cellulose, kappa-carrageenan, lambda-carrageenan, iota-carrageenan, locust bean gum, and xanthan gum) were prepared with increased dietary fiber content, enhanced emulsion stabilities, and hot/cold swelling capacities in water-oil and other aqueous systems (U.S. Patent 9,125,431).

In the U.S. Patent 6,809,197, native starch was pre-swelled or expanded in aqueous media, followed by cross-linking with sodium trimetaphosphate, reacting with octenyl succinic anhydride, and then heating to gelatinize the starch. The finished product maintained its granular structure and exhibited excellent water hydration and emulsion stabilization properties. Reversibly-swellable starch products were prepared by swelling the starch granules having a crystalline phase and cross-linking with sodium trimetaphosphate under conditions to avoid complete gelatinization. The swollen cross-linked starch granules were then heated in excess water to melt the crystalline phase. The granules exhibit a network-like structure with internal voids and capable of undergoing multiple hot or cold water swelling/drying cycles without losing the individuality of the granules. In the Australian Patent AU2012258476A1, highly cross-linked starch was prepared by reacting starch with sodium trimetaphosphate and heating above the gelatinization temperature to melt the crystalline phase. By this process, the gelatinized cross-linked starch maintains a granular morphology with resistance to alpha-amylase digestion and exhibits smooth fat-like texture when hydrated and stability to freeze-thaw treatments.

PROPERTIES OF FIBERSYM® RW

The production of Fibersym® RW involves the treatment of wheat starch with sodium trimetaphosphate (a cross-linking agent) and sodium tripolyphosphate (a substituting agent) according to the conditions given in U.S. Patent 5,855,946. This process yields a phosphorylated cross-linked RS4 wheat starch (90% minimum total dietary fiber, dry basis) in which approximately 97% of the repeat glucose units in the starch remain unchanged. The levels of chemicals used during the reaction and the residues in the final product (no more than 0.4% phosphorus) comply with Title 21 of the Code of Federal Regulations Part 172.892. The chemical name for Fibersym® is phosphated distarch phosphate. Its Chemical Abstract Service (CAS) Number is 977043-58-5 and the E-number is 1413.

The high total dietary fiber of ~90% by Fibersym® determined by AOAC Method 991.43 has been validated in vivo in a 2017 published ileostomy study at Monash University (Melbourne, Australia) (Iacovou, M., Lim, J., Maningat, C.C., Bogotyrev, A., Ly, E., Dhital, S., Gidley, M.J., Shi, Y.C., Muir, J., and Seib, P.A. 2017. In vivo digestibility of cross-linked phosphorylated (RS4) wheat starch in ileostomy subjects. Bioactive Carbohydrates and Dietary Fibre 12:25-36). In this study, ten ileostomy subjects consumed a plant-free meal containing either 26.8 g Fibersym® or 26.9 g of native wheat starch. The total starch (indigestible) in the effluents collected from the ten subjects over 24 hours showed significant amounts with Fibersym® and very minimal amounts with native wheat starch. The in vivo level of resistant starch/dietary fiber in Fibersym® was determined to be ~84%. When examined by confocal microscopy, the appearance of Fibersym® granules recovered in the effluent of one subject 4-6 h after consuming the Fibersym-containing meal showed damage at the surface of the granules due to amylolytic enzyme action in the small intestine. Thus, the Monash University study proved that AOAC Method 991.43 is the appropriate method for quantifying the resistant starch/dietary fiber content of Fibersym. For a complex food product that contains Fibersym® together with other sources of low molecular weight/high molecular weight insoluble/soluble dietary fibers, the suitable fiber method to use is AOAC Method 2001.03.

The structural features of Fibersym® can be elucidated by ^{31}P-Nuclear Magnetic Resonance (^{31}P- NMR) spectroscopy because the ^{31}P chemical shifts (signals) of model compounds are well known. As earlier stated, the add-on phosphorus in Fibersym® is low, amounting to ~0.4 %, and ^{31}P-NMR shows that the monophosphate groups add to the reacting wheat starch in two ways. The reacting phosphate either combines with one starch molecule forming phosphate monoester as shown by a multiplet ^{31}P-NMR signal observed at δ 3.5-5.0 ppm and pH~8 downfield from the ^{31}P signal of internal 50% phosphoric acid, or it combines with two starch molecules forming phosphate diester as indicated by the multiplet ^{31}P-NMR signal observed at δ 0.0-1.0 ppm. In Fibersym, the ratio of phosphate diester to monoester is approximately 2 to 1. The chemical structures of the two types of phosphate groups identified in phosphorylated cross-linked RS4 based on the ^{31}P-NMR patterns are derived from NMR studies of model phosphate-compounds of known structure.

In initial studies, a phosphorylated cross-linked RS4 wheat starch was reported by Kansas State University (Manhattan, KS) scientists to contain 93.4% total dietary fiber and 0.38% phosphorus. The RS4 product was identical to the parent native wheat starch granules in appearance as viewed by scanning electron microscopy and gave the same x-ray diffraction pattern as A-type starch. When heated at 8% starch concentration from 30°C to 95°C in a cooking viscometer like an amylograph, no pasting curve was observed because the cross-linking bonds restrict the swelling of the starch granules. Those cross-links also impart increasing resistance to alpha-amylase digestion as phosphorus incorporation increases. When analyzed by differential scanning calorimetry, the phosphorylated cross-linked wheat starch showed a melting endotherm (gelatinization) with elevated initial, peak, and conclusion temperatures (4.3°C to 10.5°C higher) versus the parent wheat starch, and showed a slight reduction in enthalpy of gelatinization (0.9 J/g lower).

Also reported in the initial study at Kansas State University is a phosphorylated cross-linked RS4 wheat starch with 72.9% total dietary fiber which exhibited a low swelling power of 2.8 g/g at 95°C and a solubility at 95°C of 0.5%. Compared to other cereal starches, the RS4 starch showed similar water vapor sorption and desorption isotherms at 25°C and at water activities below 0.8.

Upon heating a dilute aqueous slurry of Fibersym® starting at 35°C, scientists at the University of Nebraska (Lincoln, NE) found that the granular structure of Fibersym® remained essentially unchanged up to 60°C. Above 65°C, the granules became increasingly swollen, and at 75°C, the granules lost their original morphology. The enthalpies of gelatinization measured by differential scanning calorimetry were unchanged when the slurry was preheated from 35°C to 60°C but gradually disappeared when preheated between 60°C

to 85ºC, indicating the disruption of intermolecular interactions by heat. This phenomenon coincided with the gradual decrease in crystallinity and the conversion of the A-type x-ray diffraction profiles into increasingly amorphous forms within the same temperature range. It also coincided with the gradual disappearance of the endothermic peak.

Mechanism of Resistance to Enzyme Digestion

The phosphate diester linkage in Fibersym® mentioned earlier with ^{31}P-NMR signal at δ 0.0-1.0 ppm is a cross-link between starch molecules, and greatly impacts the behavior of the starch granule. Those cross-links effectively tie together all the starch molecules in a granule and produce one giant molecule that greatly restricts swelling of the modified granule in aqueous media.

The resistance of Fibersym® to digestion by α-amylase can be attributed mainly to its low degree of swelling. The unswollen cross-linked granules are impenetrable to amylolytic enzyme because enzymes are large molecules compared to the unoccupied space in the cross-linked granules. Therefore, amylolytic digestion of highly cross-linked starch is limited to the surface of granules of the modified starch. Scientists at Monash University (Melbourne, Australia), using light and confocal microscopy, examined the indigestible particulates in the output of ileostomy subjects who consumed Fibersym. Those particulates were starch granules that under microscopy showed visibly damaged surfaces caused by amylolytic enzymes in the upper GI tract of a subject. Scientists at University of Toronto (Toronto, Canada) subjected food-grade phosphorylated cross-linked RS4 wheat (Fibersym) and potato starches to successive in vitro digestion with pepsin and pancreatin-bile. The two phosphorylated cross-linked RS4 starches when digested left 74-82% indigestible residues and both showed surface erosion of granules by scanning electron microscopy. Other researchers have found that RS2-type high-amylose corn starch granules recovered from ileostomy subjects and from pigs exhibited etching and pitting of the granules.

In explaining the enzyme-resistance of phosphorylated cross-linked RS4 starch using microscopic data, one needs to consider the surface features of starch granules. Atomic force microscopy with its extremely high magnification shows nodules or blocklets protruding from the surface of starch granules. Those surface nodules are readily accessible and susceptible to amylase attack, and the nodules explain the low level of digestion of Fibersym. Phosphate cross-links strongly restrict granule swelling during cooking which hinders entry of amylase into the inner zone of granules and accounts for much of the resistance. The phosphate groups on and between starch chains can also sterically hinder the formation of the molecular complex between the amylase and starch, resulting in inhibition. Surface pores and channels commonly seen on wheat starch granules are likely blocked or obstructed by phosphate groups, thereby hindering diffusion of amylase enzymes.

Methodology for Measuring Resistant Starch (Dietary Fiber) Content of Fibersym® RW

Fibersym® RW delivers a minimum total dietary fiber of 90 % (dry basis) using the in vitro conditions specified in AOAC Method 991.43. The fiber exists primarily as insoluble fiber. A human clinical study at Monash University (Melbourne, Australia) using ileostomy subjects showed 84% in vivo resistant starch in Fibersym, which validates the AOAC Method 991.43 or AOAC Method 985.29 as the preferred in vitro fiber method of use.

When a resistant starch is present in a food as in the case of Fibersym, the in vitro enzymatic hydrolysis conditions must be set to remove digestible starch, but leave behind resistant starch. In the case of AOAC Method 2002.02 for measuring resistant starch content of foods, the resistant starch residue is directly measured by solubilizing the resistant starch fraction and converting the solubilized starch to glucose with amyloglucosidase followed by spectrophotometric quantitation of glucose. However, the resistant starch residue from a phosphorylated cross-linked RS4 starch like Fibersym® is not soluble in either of the two solvents used namely, 2M KOH and dimethylsulfoxide. In order to resolve this analytical problem, scientists at Kansas State University developed a new improved method to directly measure the resistant starch content in phosphorylated cross-linked starch. The method involves digestion with pancreatic alpha-amylase and amyloglucosidase, ethanol (99%) precipitation of the resistant starch residue, followed by washing the residue three times with 50% ethanol, and then recovering the residue by centrifugation. The residue is treated with 2M KOH, neutralized with 2M HCl followed by the addition of 99% ethanol, and centrifugation to remove the salts and recover the residue. The residue is digested twice with thermostable alpha-amylase, incubated with amyloglucosidase, and finally the glucose released is quantified spectrophotometrically, which is a measure of the resistant starch content. This procedure converted 95.3% of the enzyme-resistant residue to glucose.

GLUTEN-FREE FIBERSYM® RW

In August 2013, FDA issued the final rule on gluten-free labeling of foods under the Food Allergen Labeling and Consumer Protection Act of 2004 to provide truthful and accurate information to individuals with celiac disease and to those who have gluten sensitivity or intolerance. The term "gluten-free" is defined to mean that the food contains less than 20 parts per million of gluten (Food and Drug Administration. 2013. Food Labelling: Gluten-Free Labeling of Foods. Federal Register, Vol. 78, No. 150, pages 47154-47179, August 5). A food that fails to meet a "gluten-free" claim will be deemed misbranded.

Fibersym® can be made gluten-free and used as an ingredient to enhance the fiber content and lower calories of gluten-free food products. During its manufacture, a purified stream of wheat starch slurry coupled with the conditions of the modification process bring the residual gluten content to less than the regulatory limit of 20 parts per million based on R-Biopharm RIDASCREEN® assay. This particular assay has a lower limit of quantitation of 5 parts per million gluten and is normally performed by the Food Allergy Research and Resource Program at University of Nebraska (Lincoln, Nebraska) and other commercial testing laboratories.

The historical data on the gluten content of Fibersym® demonstrate that it can be produced with gluten levels below the FDA regulatory limit of 20 parts per million. Despite Fibersym's labeling declaration as "modified wheat starch" which contains the word "wheat", it can be considered a gluten-free fiber source. Fibersym, as a gluten-free fiber ingredient and certified Non-GMO Project Verified, will help bakers and food formulators make informed decisions about incorporating it in gluten-free food products.

PHYSIOLOGICAL BENEFITS OF FIBERSYM®RW

Dietary fibers, in general, promote a number of physiological benefits, including the following: decreased intestinal transit time; fermentation by colonic bacteria; reduced blood total and/or LDL cholesterol; and reduced post-prandial blood glucose and/or insulin. All of these benefits can decrease the risk of lifestyle diseases such as obesity, diabetes, hypertension, cardiovascular disease and cancer. Resistant starch, which is included in the definition of dietary fiber, demonstrates similar physiological benefits.

However, in the new Nutrition Facts Rule issued on May 27, 2016, FDA identified the following recognized physiological effects of dietary fiber that it considers to be beneficial effects: lowering of total/LDL cholesterol levels; lowering of post-prandial glucose and/or insulin levels; reduction of gut transit time and improving laxation (fecal output); reduction of blood pressure; increased mineral absorption in the intestinal tract; and increased satiety associated with reduced energy intake and with possible associated outcomes on body weight. Although an isolated or synthetic non-digestible carbohydrate may provide more than one beneficial physiological effect to human health, the agency considers only one beneficial physiological effect is necessary to meet the new dietary fiber definition. FDA concluded that colonic fermentation and short-chain fatty acid production and modulation of the colonic microflora are processes that may be associated with a physiological endpoint, rather than physiological endpoints themselves, and therefore are not, on their own, beneficial physiological effects.

According to FDA's guidance document for the food industry, clinical studies should provide an appropriate control group and statistical analysis so that it is possible to determine whether an effect on a particular physiological endpoint was due to the isolated or synthetic non-digestible carbohydrate or due to unrelated and uncontrolled extraneous factors of the study. An appropriate control group represents study subjects who did not receive or consumed a lower amount of the added non-digestible carbohydrate of interest. When the intervention study involves providing subjects with a whole food, along with an added non-digestible carbohydrate, the experimental and control diets should be similar enough that the relationship between the added non-digestible carbohydrate and beneficial physiological effect can be evaluated. The composition of the experimental and control diets should be similar for all food components, except for the added non-digestible carbohydrate of interest. In addition, the level of available (digestible) carbohydrate must be the same between the control food and the test food.

When evaluating the strength of scientific evidence for the role of foods/nutrients in their beneficial physiological effects to human health, FDA uses generally-recognized scientific reviews, such as the type, number and size of the studies, whether limited evidence showing an effect has been replicated, and whether the findings are consistent in showing a statistically significant effect ($p<0.05$) on the health-related endpoint being evaluated.

BLOOD GLUCOSE AND INSULIN REGULATION

A number of human clinical studies have evaluated the effect of Fibersym®RW on postprandial blood glucose and insulin response. These studies demonstrated a statistically significant decrease in glycemic and/or insulinemic response when consuming food products containing Fibersym® as compared with food products without added Fibersym. A lower glycemic response is associated with beneficial outcomes as it corresponds to a reduced insulin release, enhanced blood glucose control, and a reduction in blood lipids, which may help reduce risk factors associated with heart disease and type 2 diabetes. Blood glucose control is vital in preventing secondary complications such as renal disease, retinopathy, neuropathy, and atherosclerosis. According to FDA, a lower insulin response after a meal, without a higher glycemic response among healthy subjects, is a beneficial physiological effect because less insulin is required to achieve a similar, or lower, glycemic effect. Attenuation of postprandial insulin response is associated with a reduced risk of coronary heart disease.

In a controlled randomized crossover clinical trial at Kansas State University (Manhattan, Kansas) using the standard glycemic index protocol, 13 healthy human subjects (females = 7; males = 6; age = 27 ± 5 yr; BMI = 25 ± 3 kg/m2) were fed two nutritional bars, as well as a control dextrose drink, after a 12-hour overnight fast. Puffed wheat was formulated in a nutritional bar and Fibersym® was formulated in a second nutritional bar by totally replacing puffed wheat in the formula (**Table 1**). All the other ingredients were kept identical. The two bar samples and the glucose drink each delivered 50 grams of available carbohydrates (**Table 2**). Blood glucose and insulin levels were monitored for two hours after the samples were consumed.

Table 1. *Ingredients and their concentrations by relative weight (% total) in the test bars.*

Ingredient	PWB	$RS4_{XL}$
Puffed Wheat[a]	34	--
Resistant Starch type 4[b]	--	34
Corn Syrup[c]	20	20
Wheat Germ[d]	18	18
Brown Sugar[e]	11	11
Water[f]	10	10
Gum Acacia[g]	6	6
Panodan 150K[h]	1	1

[a] *Quaker Oats*
[b] *Fibersym®RW; MGP Ingredients, Inc.*
[c] *Karo light corn syrup*
[d] *Kretschmer Original Toasted*
[e] *C&H Pure Cane Sugar, golden brown*
[f] *Tap water (Manhattan, Kan)*
[g] *TIC Gums*
[h] *Danisco*

Table 2. *Nutrient composition of each treatment per dose (GLU = 198 mL; PWB = 65 g; RS4 = 80 g).*

Nutrient	GLU[a]	PWB[b]	RSX^{b}_{XL}	Δ[e]
Total Energy (kcal)	200	261	326	(65 kcal, 125%)
Carbohydrate (g)				
Total	50	56	71	(15 g, 127%)
Available[c]	50	51	51	(0 g, 0%)
Total Dietary Fiber (g)[d]	--	5	20	(15 g, 400%)
Fat (g)	--	1	2	(1 g, 200%)
Protein (g)	--	7	6	(1 g, 86%)

[a] *Glucose tolernance test beverage (Sun-Dex, Fisher Scientific, Houston, Tex)*
[b] *Crude nutrient composition was determined by proximate analysis (total energy, total fat, total protein, total carbohydrate).*
[c] *Derived by subtracting total dietary fiber from total carbohydrate.*
[d] *Dietary fiber analysis was performed by Medallion Laboratories (Minneapolis, Minn).*
[e] *Difference (subtraction value, % value) between bars.*

Tables above are reproduced with permission from *Al-Tamimi, E.K., Seib, P.A., Snyder, B.S., and Haub, M.D. 2010. Consumption of cross-linked resistant starch (RS4XL) on glucose and insulin responses in humans. J. Nutr. Metab. 2010: Article ID 651063.*

BLOOD GLUCOSE AND INSULIN REGULATION ▸ *Continued*

The peak glucose (5.40 mmol/L) and insulin (162.3 pM) concentrations were significantly lower ($p<0.05$) for the Fibersym® bar compared to dextrose (7.30 mmol/L; 344 pM) and the puffed wheat bar (6.33 mmol/L; 211.5 pM) (**Table 3**). The 2-hour incremental areas under the curve (iAUC) for glucose (28 mmol/L x 2 hr) and insulin (3659 pM x 2 hr) were significantly lower ($p<0.05$) following ingestion of the bar containing Fibersym® compared to the puffed wheat bar (84 mmol/L x 2 hr; 8758 pM x 2 hr) and the control dextrose drink (140 mmol/L x 2 hr; 17575 pM X 2 hr). Thus, with matching 50 g available carbohydrate, postprandial blood glucose and insulin levels were significantly lowered following the consumption of the Fibersym® bar compared to the puffed wheat bar and the control dextrose drink (**Fig. 1**).

In a second clinical study, researchers at Glycemic Index Laboratories, now Inquis Clinical Research, (Toronto, Canada) used a controlled randomized double-blind clinical study involving 15 healthy human subjects (females = 10; males = 5; age = 32 ± 11 yr; BMI = 24.9 ± 5 kg/m2) who consumed either a cookie containing Fibersym® or a control cookie, both of which contained 40 g available carbohydrate. The peak blood glucose concentration at 30 and 45 min was significantly lower ($p<0.05$) following consumption of the Fibersym® cookie compared to the control cookie. The 2-hour iAUC for insulin was significantly lower ($p<0.02$) following consumption of the Fibersym® cookie compared to the control cookie. Thus, this study demonstrated that Fibersym® lowers post-prandial blood glucose and insulin levels when controlling for the amount of available carbohydrate in the test food and the control food.

Figure 1. *Depiction of the glucose (**a**) and insulin (**b**) responses over two hours following the consumption of each (GLU, PWB, and RS4) treatment. Values represent each mean ± SE. A; significant difference with PWB; B: significant difference with RS4.*

Source: *Haub et al 2010.* Used with permission.

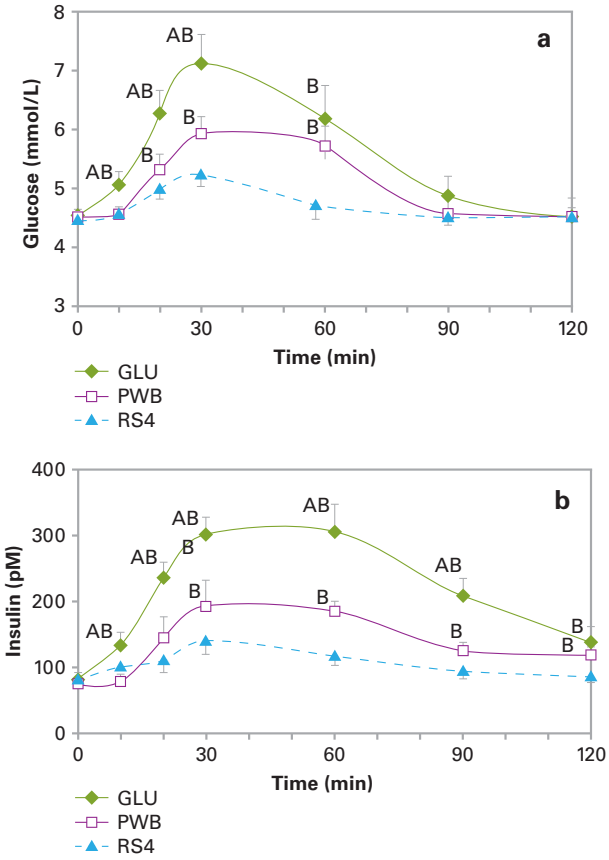


Table 3. *Values for the incremental areas under the curves of glucose and insulin concentrations during each trial. Mean ± SE; different letters within a row indicates significant difference (P < 0.05).*

	GLUC	PWB	$RS4_{XL}$
Glucose			
iAUC (mmol/L · 2 hr)	140 ± 31^{A}	84 ± 17^{B}	28 ± 11^{C}
Peak (mmol/L)	7.30 ± 0.5^{A}	6.33 ± 0.3^{B}	5.40 ± 0.2^{C}
Increase (%)	60.5 ± 10^{A}	42.7 ± 6^{A}	20.4 ± 3^{B}
Insulin			
iAUC (pM · 2 hr)	$17{,}575 \pm 2{,}236^{A}$	$8{,}758 \pm 1{,}132^{B}$	$3{,}659 \pm 974^{C}$
Peak (pM)	344 ± 36.7^{A}	211.5 ± 20.1^{B}	162.3 ± 22.6^{C}
Increase (%)	335 ± 53.2^{A}	243.0 ± 49.3^{B}	126.3 ± 45.8^{C}

Source: *Al-Tamimi et al 2010.* Used with permission.

In a follow-up clinical trial on nutritional bars, scientists at Kansas State University tested two levels of matching available carbohydrate on post-prandial blood glucose and insulin levels following ingestion of a Fibersym® bar and a control bar. In a controlled randomized crossover trial, 15 healthy human subjects were fed either a nutritional bar containing Fibersym® or a control nutritional bar, both of which contained 50 g available carbohydrate or 30 g available carbohydrate. At 50 g available carbohydrate, the peak blood glucose concentration following consumption of the Fibersym® bar was significantly less (p=0.01) than following consumption of the control bar. Furthermore, the 2-hour iAUC for blood glucose was significantly lower (p=0.03) following consumption of the Fibersym® bar compared to the control bar, and the baseline to peak blood glucose concentration was significantly lower following consumption of the Fibersym® bar compared to the control bar (p=0.02). At 30 g available carbohydrate, peak blood glucose concentration, baseline to peak blood glucose concentration, and 2-hour iAUC trended lower for the Fibersym® bar compared to the control bar, but did not reach statistical significance.

At 50 g and 30 g available carbohydrate dosage, the 2-hour iAUC for insulin was significantly less following consumption of the Fibersym® bar than following consumption of the control bar ($p<0.01$ for both amounts of available carbohydrate). At 50 g and 30 g available carbohydrate dosage, the peak blood insulin level was significantly lower following consumption of the Fibersym® bar than following consumption of the control bar (p=0.01 for 50 g available carbohydrate, p=0.04 for 30 g available carbohydrate). At 50 g and 30 g available carbohydrate dosage, the baseline to peak blood insulin level was significantly lower following consumption of the Fibersym® bar than following consumption of the control bar (p=0.02 for 50 g available carbohydrate, p=0.03 for 30 g available carbohydrate). At 50 g available carbohydrate, the blood insulin level after 30 min was significantly lower following consumption of the Fibersym® bar than following consumption of the control bar (p=0.03).

The results of the above clinical studies are summarized in **Table 4**. The strength of scientific evidence supports that Fibersym® provides physiological effects that are beneficial to human health.

In another clinical study at Kansas State University, eleven healthy human subjects (females = 7; males = 4; age = 24 ± 4 yr; height = 1.65 ± 0.07 m; weight = 63.7 ± 13.1 kg; BMI = 23.2 ± 3.8 kg/m2) were recruited and asked to consume on three separate occasions, after a

Table 4. *Summary of three human clinical studies on glycemic and insulinemic responses of Fibersym® RW demonstrating its beneficial physiological effects to human health.*

Clinical Study	Study Design	Available Carbohydrate	Compliance with FDA Guidance for Industry	Physiological Effect
Al-Tamimi et al, 2010	Controlled, randomized, crossover design involving 13 healthy subjects	Matching 50 g available carbohydrate for Fibersym®bar and Control bar	Compliant	Significantly lowers postprandial blood glucose level based on peak glucose and iAUC; Significantly lowers postprandial blood insulin level based on peak insulin and iAUC
Glycemic Index Laboratories (now Inquis Clinical Research) study 2018	Acute, double blind, randomized, controlled design involving 15 healthy volunteers	Matching 40 g available carbohydrate for Fibersym cookie and Control cookie	Compliant	Significantly lowers postprandial blood glucose and insulin levels based on peak glucose and iAUC, respectively
Kansas State University study, 2019	Controlled, randomized, crossover design involving 15 healthy subjects	Treatment 1 with matching 30 g available carbohydrate for Fibersym®bar and Control bar; Treament 2 with matching 50 g available available carbohydrate for Fibersym®bar and Control bar	Compliant	Significantly lowers postprandial blood glucose and insulin levels at 50 g available carbohydrate based on iAUC and peak glucose/insulin; Significantly lowers postprandial insulin level at 30 g available carbohydrate based on iAUC and peak insulin

BLOOD GLUCOSE AND INSULIN REGULATION ▸ *Continued*

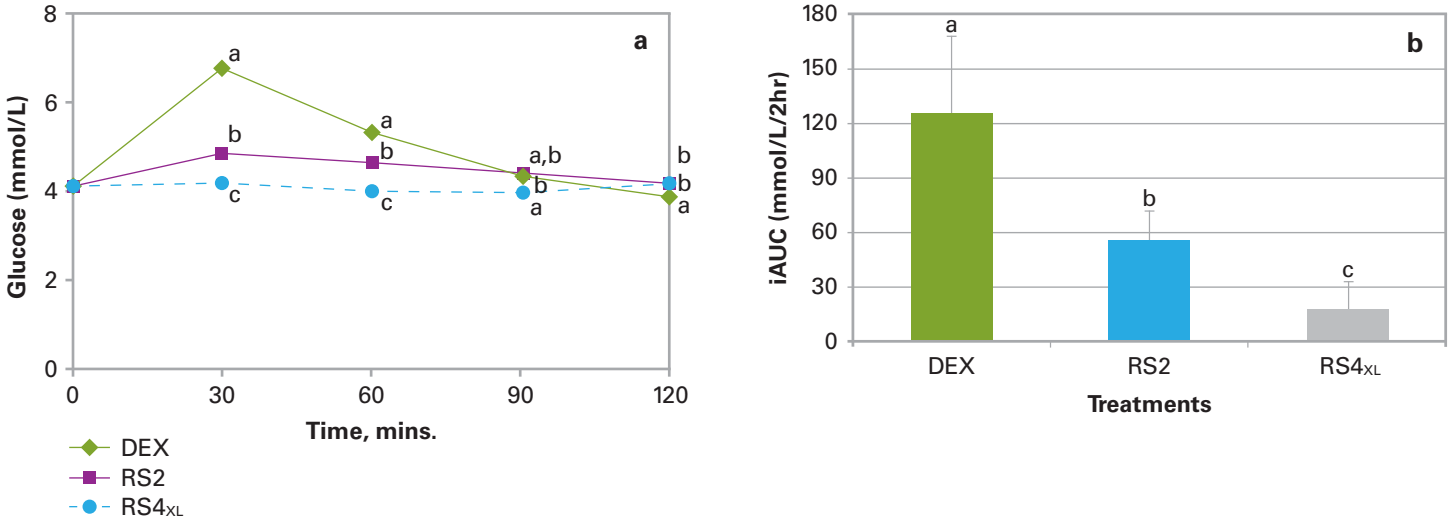
10-12 hour fasted state, 30 grams of either dextrose, RS2 resistant starch (Hi-Maize 260) or RS4 resistant starch (Fibersym®) dispersed or dissolved in 178 ml water. Finger-prick capillary blood samples were collected before and over the following two hours. Blood glucose levels were immediately measured in duplicate using an automated blood glucose analyzer.

The peak in glucose response for dextrose and Hi-Maize 260 occurred at 30 minutes, while the glucose peak during the Fibersym® treatment did not occur until 120 minutes, indicating a slower digestion rate for Fibersym® (Fig. 2a). Both Fibersym® and Hi-Maize 260 significantly decreased ($p<0.05$) the incremental area under the curve compared with dextrose (Fig. 2b). The glucose response with Fibersym® was significantly decreased compared to Hi-Maize 260. The relative glycemic responses were 100% for dextrose, 34.9±11% for Hi-Maize 260, and 11.3±10% for Fibersym, respectively, indicating a more effective attenuation by Fibersym® of post-prandial blood glucose compared to Hi-Maize 260.

In a clinical study conducted at Iowa State University (Ames, Iowa), a breakfast meal was used, which consisted of an orange juice beverage and a muffin made either from all-purpose flour (control) or a muffin made from a blend of 60/40 blend of all-purpose flour/Fibersym®(test). Including the orange juice beverage, the Fibersym® breakfast meal provided 693 kcal, 16 g protein, 102 g available carbohydrates, 24 g fat and 26 g fiber. The control breakfast meal delivered 777 kcal, 16 g protein, 122 g available carbohydrates, 24 g fat and 2 g fiber. The level of available carbohydrate in the two meals was high (122 g for control breakfast meal and 102 g for Fibersym® breakfast meal). Statistical analysis of the results revealed lower blood insulin ($p<0.05$) after the Fibersym® breakfast meal consumption compared with the control breakfast meal. No statistically significant effect ($p>0.05$) was found for blood glucose after consuming the Fibersym® breakfast meal compared to the control breakfast meal.

The above studies demonstrate that Fibersym® could be an important ingredient in foods for reducing the risk of type 2 diabetes and other health issues related to blood glucose and insulin regulation.

Figure 2. *The glucose responses to 30 g of carbohydrate from three treatments (DEX, RS2 and $RS4_{XL}$). Panel (**a**) depicts the glucose changes over time, while panel (**b**) depicts the incremental area under the glucose curve. Data presented are mean ± SE; significance was set at P < 0.05; and, different letter indicate difference between treatments.*



Reproduced with permission from *Haub, M.D., Hubach, K.L., Al-Tamimi, E.K., Ornelas, S., and Seib, P.A. 2010. Different types of resistant starch elicit different glucose responses in humans. J. Nutr. Metab. 2010: Article ID 230501.*

ROLE IN CHOLESTEROL REDUCTION

Two human clinical studies at South Dakota State University (Brookings, SD) have evaluated the effect of Fibersym® on total blood cholesterol levels. The studies (registered at clinicaltrials.gov as NCT01887964) were conducted within Hutterite communities in eastern South Dakota. The Hutterites, also called Anabaptists, are an interesting population because they are a culturally homogeneous group of Caucasians of Central European ancestry. They practice a communal lifestyle and have relatively small interpersonal differences in diet due to centralized meal planning, kitchen, and dining practices. Even their mealtime is exactly the same every day. Men and women gather in the common dining hall and the dining starts and ends at the ring of bells. The two studies were conducted on relatively isolated communities that live and eat together as a colony. They were both cross-over intervention studies that evaluated the health benefits of using Fibersym® to prepare the daily meals eaten by the entire colony, and therefore these results translate directly to "real-world" consumption of this ingredient. Each of these studies demonstrated a statistically significant reduction in total blood cholesterol levels following extended consumption of food products prepared with flour containing Fibersym® as compared to consumption of food products prepared with standard flour.

In a 26-week long double-blind (participants investigators), placebo-controlled, cluster cross-over intervention study, two colonies of Hutterites were used as subjects in evaluating the effects of dietary Fibersym® consumption in healthy participants both with (46.5% of the recruited subjects) and without metabolic syndrome. The 26-week study consisted of two 12-week intervention periods (one each for Fibersym-Flour and Control Flour) with a two-week washout period in between. Exclusion criteria for subjects included pregnancy, lactation, long-term antibiotic therapy, immune-compromised, cancer and other conditions that would affect the ability to provide informed consent or comply with the protocol.

The Control wheat flour and a blend of 30% Fibersym/70% wheat flour, with nutrient composition shown in **Table 5**, were provided to these two colonies for use in the preparation of foods such as bread, noodles, maultaschen, and dumplings. Participants (total = 83) consumed foods containing Fibersym-Flour or Control Flour ad libitum in a free-living, domestic environment. All data and biospecimens were collected onsite from both colonies at baseline, 12, 14, and 26 weeks, except body composition analysis, which was omitted at 24 weeks.

Fibersym-Flour consumption had a significant lowering effect ($p \leq 0.05$) of total cholesterol for healthy subjects without metabolic syndrome. All participants (healthy individuals with

Table 5. *Nutrient composition (g/100 g) of control flour and 30% Fibersym/70% flour blend.*

Nutrient	Control Flour	Fibersym-Flour Blend
Water	*13.4*	*12.5*
Protein	*11.0*	*7.9*
Carbohydrate	*73.5*	*77.8*
Total Fat	*1.7*	*1.3*
Saturated Fat	*0.2*	*0.2*
Monounsaturated Fat	*0.1*	*0.1*
Polyunsaturated Fat	*0.7*	*0.6*
Trans-Fat	*0*	*0*
Total Dietary Fiber	*2.4*	*25.7*
Sugars	*0.3*	*0.2*
Ash	*0.5*	*0.6*
Calcium (mg/100 g)	*24.0*	*50.4*
Sodium (mg/100 g)	*2.0*	*91.4*
Vitamin C (mg/100 g)	*0*	*0.4*
Calories (kcal)	*361.0*	*266.8*

Adapted from *Nichenametla, S.N., Weidauer, L.A., Wey, H.E., Beare, T.M., Specker, B.L., and Dey, M. 2014. Resistant starch type 4-enriched diet lowered blood cholesterol levels and improved body composition in a double blind controlled cross-over intervention. Mol. Nutr. Food Res. 00, 1-5 (DOI 10.1002/mnfr.201300829).*

ROLE IN CHOLESTEROL REDUCTION ▸ *Continued*

or without metabolic syndrome) exhibited lower (p≤0.05) total cholesterol, HDL cholesterol and non-HDL cholesterol post Fibersym-Flour consumption.

The results also showed that Fibersym-Flour consumption, compared with Control Flour, resulted in 7.2% lower (p=0.002) mean total cholesterol, 5.5% lower (p=0.04) non-HDL cholesterol and a 12.8% lower (p<0.001) HDL cholesterol in the healthy group with metabolic syndrome.

The second study involved 20 healthy individuals with metabolic syndrome (12 females and eight males, aged 32-77) from two Hutterite colonies in eastern South Dakota. Participants consumed food products made with Control Flour or Fibersym-Flour (30%/70% blend) ad libitum in a free-living, domestic environment with no dietary restrictions imposed as described in the above first study. Blood and stool samples were collected and height, weight, waist circumference and blood pressure of participants were measured.

Reduction in plasma total cholesterol (p<0.001), non-HDL cholesterol (p<0.01) and HDL cholesterol (p<0.01) after Fibersym-Flour consumption was observed when compared with the Control Flour group (**Table 6**). The same reduction in plasma total cholesterol (p=0.01), non-HDL cholesterol (p=0.03) and HDL cholesterol (p=0.001) after Fibersym-Flour consumption was observed when compared with the baseline group. Non-HDL cholesterol comprises LDL +VLDL cholesterol, both of which are unhealthy.

Table 6. *Impact of Fibersym® intervention on biological parameters as reflected on p-v Fibersym/70% flour blend.*

Parameter	
Waist	
% Body Fat	
Total Cholesterol	
HDL Cholesterol	
LDL Cholesterol	
Non-HDL Cholesterol	
IL6	
Adiponectin	

Adapted with permission from *Upadhyaya, B., McCormack, L., Fardin-Kia, A.R., Juen Impact of dietary resistant starch type 4 on human gut microbiota and immunometabo creativecommons.org/licenses/by/4.0*

IMPACT ON GASTROINTESTINAL HEALTH

Physical activity, portion control and wholesome foods define a person's healthy lifestyle. While the first two items are behavioral in nature, the third one can be designed in most foods. Food developers have previously associated the end-use quality of foods from the moment of growing the crop to the time the processed food is served and consumed – the so-called "From Farm to the Table" pathway. Recently, with health and wellness resonating well with consumers, this connection is being transformed into a "From Farm to Fitness" pathway.

The lower gut or gastrointestinal tract (commonly called the large bowel or colon) was originally thought to be an organ in the human body for processing waste (undigested residues) after food is consumed. There was limited awareness that human health may lie in the gut as researchers have recently uncovered.

The human gut harbors extremely dense and highly diverse microbial communities called microbiota consisting of over 500 different bacterial species whose cell numbers exceed 100 billion per gram. The gut is a complex microbial ecosystem as it is a nutrient-rich, open system with a constant temperature and continuous turnover. For survival, these obligate anaerobic microorganisms have to reproduce at a rate sufficient to avoid washout or have an ability to attach to or colonize host tissues. The makeup of microorganisms inhabiting an individual's gastrointestinal tract represents a fingerprint, which differentiates him or her from another individual. The bacteria that colonize the large intestine have access only to the dietary residue that evades digestion by host enzymes in the upper gut or small intestine.

Microorganisms in the lower gut can help with digestion, stimulate cell growth, strengthen the immune system, break down toxins and/or protect against some diseases. In particular, Bifidobacteria are considered health-promoting microorganisms as they demonstrate anti-tumor activity and have demonstrated prophylactic and therapeutic benefits for colon cancer and chronic inflammation in animals. In addition, Bifidobacteria have been shown to be associated with benefits in glucose tolerance, insulin response and cholesterol metabolism in animals. Other microorganisms, however, function differently as they may contribute to Western lifestyle diseases such as obesity, type 2 diabetes, coronary heart disease, inflammatory bowel disease or cancer. The key to good health is promoting the proliferation of beneficial microorganisms and reducing the unfriendly ones.

values post control flour vs 30% Fibersym/70% flour blend and baseline vs post 30%

p-Value: Post Control Flour vs Post Fibersym-Flour Blend	p-Value: Baseline vs Post Fibersym-Flour Blend
0.06	0.02
0.05	NS
<0.001	0.01
<0.01	0.001
0.06	0.06
<0.01	0.03
NS	0.04
0.02	<0.01

emann, R., Nichenametla, S., Clapper, J., Specker, B., and Dey, M. 2016. lic functions. Scientific Reports 6:28797; doi:10.1038/srep28797. http://

Recently, consumers have been exposed to food products that are infused with a cocktail of friendly bacteria (called probiotics) such as yogurts, smoothies, snack bars, cereal products and even pills. These products are touted to regulate digestive health. Understanding the role of gut microorganisms helps consumers adjust their eating practices and assists food scientists in designing or tailoring foods that can address health concerns.

There is prevailing evidence that the microbiota responds to changes in the diet, in particular to the type and quantity of dietary carbohydrates. Resistant starches escape digestion and absorption in the human small intestine and reach the large bowel where they serve as substrate for fermentation by the large bacterial populations residing there. Scientists have discovered that fermentation of resistant starch in the colon has beneficial health effects to the host. Resistant starch plays an important role in colonic fermentation, short-chain fatty acid production, and positive modulation of colonic microflora.

A clinical study conducted at University of Nebraska determined the impact of Fibersym® on gut microbiota composition and metabolism. The study determined if resistant starch shows prebiotic properties in vivo and to gauge the impact on gastrointestinal microbial composition and metabolism. A prebiotic is defined as a non-viable food component, ingredient or supplement that selectively modulates the microbiota of the digestive ecosystem, and confers benefits upon the host's well-being and health. The Nebraska study characterized the effects of two types of resistant starch compared to a control starch on the composition and metabolism of gut microbiota in human subjects using modern, culture-independent molecular techniques and analytical methods such as polymerase chain reaction and denaturing gradient gel electrophoresis.

Ten healthy, non-vegetarian human subjects (5 males and 5 females) between 23-38 years of age were recruited for the study. None of the subjects had been on antibiotics within 3 months prior to the beginning of the study or during the study. The study was conducted over a 17-week period during which the subjects maintained their usual lifestyles while consuming as a supplement snack crackers formulated with RS4 wheat starch (Fibersym), RS2 high-amylose corn starch (Hi-Maize 260), or native wheat starch (control). The snack crackers were prepared by the American Institute of Baking International in Manhattan, Kansas.

Both types of resistant starch were well tolerated by the subjects at a dose of 33 grams of fiber per day for three weeks. The population or cell count of *Bifidobacteria* was significantly higher when Fibersym® and Hi-Maize 260 crackers were consumed as compared to bifidobacterial population during consumption of the control crackers. On average, Fibersym® crackers increased the amount of *Bifidobacteria* by 350%, while Hi-Maize 260 crackers increased bifidobacterial counts by 210%. The specie of *Bifidobacteria* that increased in population was identified to be *Bifidobacterium adolescentis*, a friendly, non-pathogenic organism that resides in the gut of healthy humans. This microorganism has been reported in the literature to synthesize various B vitamins that are beneficial to the nutritional health of humans. As a producer of the vitamin folate in the colon, *Bifidobacterium adolescentis* protects the colon against inflammation and cancer. It is also capable of metabolizing carbohydrate that is later converted by other microbes to short-chain fatty acids such as acetate, propionate and butyrate to be used as energy sources.

It took 2-3 weeks of consuming Hi-Maize 260 crackers before the increase in cell counts of *Bifidobacterium adolescentis* became obvious, while Fibersym® crackers showed a marked response within the first week of ingestion. Thus, the positive effect occurs faster for Fibersym® compared to Hi-Maize 260. A follow-up feeding study confirmed that administration of Fibersym crackers resulted in a significant population increase

IMPACT ON GASTROINTESTINAL HEALTH ▸ *Continued*

of *Bifidobacterium adolescentis* within 2-4 days after consuming the crackers. This study provided evidence that both Fibersym® and Hi-Maize 260 show bifidogenic potential. Fibersym® had a higher bifidogenic effect and the increase in bifidobacterial numbers was more immediate.

Fibersym® also caused phylum-level changes in gut bacteria resulting in a significant increase in Actinobacteria (e.g. *bifidobacteria*) and Bacteroidetes as opposed to a decrease in the level of Firmicutes (**Table 7**). A low Firmicutes/Bacteroidetes ratio has been reported to be associated with lean human subjects, which is linked to a decreased capacity for energy harvest as it

Table 7. *Relative abundance of bacterial species (log fold change) in the RS4 group compared with the CF group post intervention (n = 19). Significant compositional variation between the two groups before the intervention was previously ruled out.*

Proportion of bacterial taxa expressed in percentage (Mean ± SD)

	RS2[1]	RS4[1]	Control[1]	Baseline[2]	Washout[3]	P-Value[4]
PHYLUM						
Firmicutes	**75.9 ± 13.4**	*65.6 ± 15.0*	**79.6 ± 9.6**	78.2 ± 7.5	78.1 ± 8.5	0.0010
Bacteroidetes	*10.1 ± 6.6*	**16.3 ± 9.7**	*10.4 ± 6.9*	12.7 ± 6.5	12.2 ± 5.8	0.0028
Actinobacteria	6.1 ± 6.4	**11.4 ± 12.5**	*4.1 ± 3.1*	3.1 ± 2.5	4.1 ± 3.2	0.0334
FAMILY						
Bifidobacteriaceae	5.8 ± 6.0	**11.1 ± 11.7**	*3.0 ± 2.5*	2.1 ± 1.7	2.8 ± 2.2	0.0262
Porphyromonadaceae	*0.6 ± 1.0*	**3.4 ± 1.9**	*0.5 ± 0.3*	0.6 ± 0.4	0.5 ± 0.4	0.0002
Ruminococcaceae	**24.8 ± 13.6**	*16.7 ± 7.4*	**23.2 ± 9.7**	19.3 ± 7.4	20.7 ± 7.6	0.0031
Erysipelotrichaceae	3.1 ± 2.8	*2.6 ± 2.6*	**3.9 ± 3.2**	4.7 ± 4.9	3.9 ± 3.1	0.0279
GENUS						
Faecalibacterium	9.7 ± 4.4	*7.8 ± 3.4*	**10.8 ± 4.7**	8.4 ± 4.2	8.8 ± 2.9	0.0336
Parabacteroides	*0.6 ± 1.0*	**3.4 ± 1.9**	*0.4 ± 0.5*	0.5 ± 0.3	0.5 ± 0.4	0.0002
Bifidobacterium	4.5 ± 4.9	**8.9 ± 10.2**	*2.2 ± 1.7*	1.5 ± 1.3	2.1 ± 1.6	0.0342
Dorea	*1.7 ± 1.2*	*1.6 ± 1.2*	**3.0 ± 2.0**	2.9 ± 2.2	2.7 ± 2.0	0.0030
SPECIES (OTUs)						
B. adolescentis	3.7 ± 4.5	**7.9 ± 10.3**	*1.7 ± 1.9*	1.5 ± 1.2	1.8 ± 1.3	0.0347
P. distasonis	*0.2 ± 0.4*	**1.5 ± 1.0**	*0.2 ± 0.1*	0.2 ± 0.1	0.2 ± 0.2	0.0002
R. bromii	**4.1 ± 5.1**	*1.2 ± 1.3*	2.6 ± 3.2	1.0 ± 1.1	2.0 ± 1.5	0.0479
F. prausnitzii	4.8 ± 2.6	*3.6 ± 2.0*	**5.6 ± 3.1**	4.2 ± 2.8	4.2 ± 2.4	0.0160
E. rectale	**8.3 ± 7.1**	*3.4 ± 2.3*	4.9 ± 4.0	5.4 ± 2.9	4.7 ± 2.0	0.0301
D. formicigenerans	1.2 ± 1.0	*1.0 ± 1.1*	**2.2 ± 1.6**	2.3 ± 1.8	1.9 ± 1.7	0.0140
C. clostridioforme	2.6 ± 2.4	**3.4 ± 2.5**	*1.2 ± 0.8*	1.4 ± 1.3	1.5 ± 1.2	0.0126
Clostridiales spp.	*0.3 ± 0.6*	**0.9 ± 0.9**	0.7 ± 0.8	0.2 ± 0.4	0.8 ± 0.7	0.0322

[1]*The bacteria populations are averages of all three time points of feeding periods.*

[2]*The bacteria populations are averages of the two time points of the baseline period.*

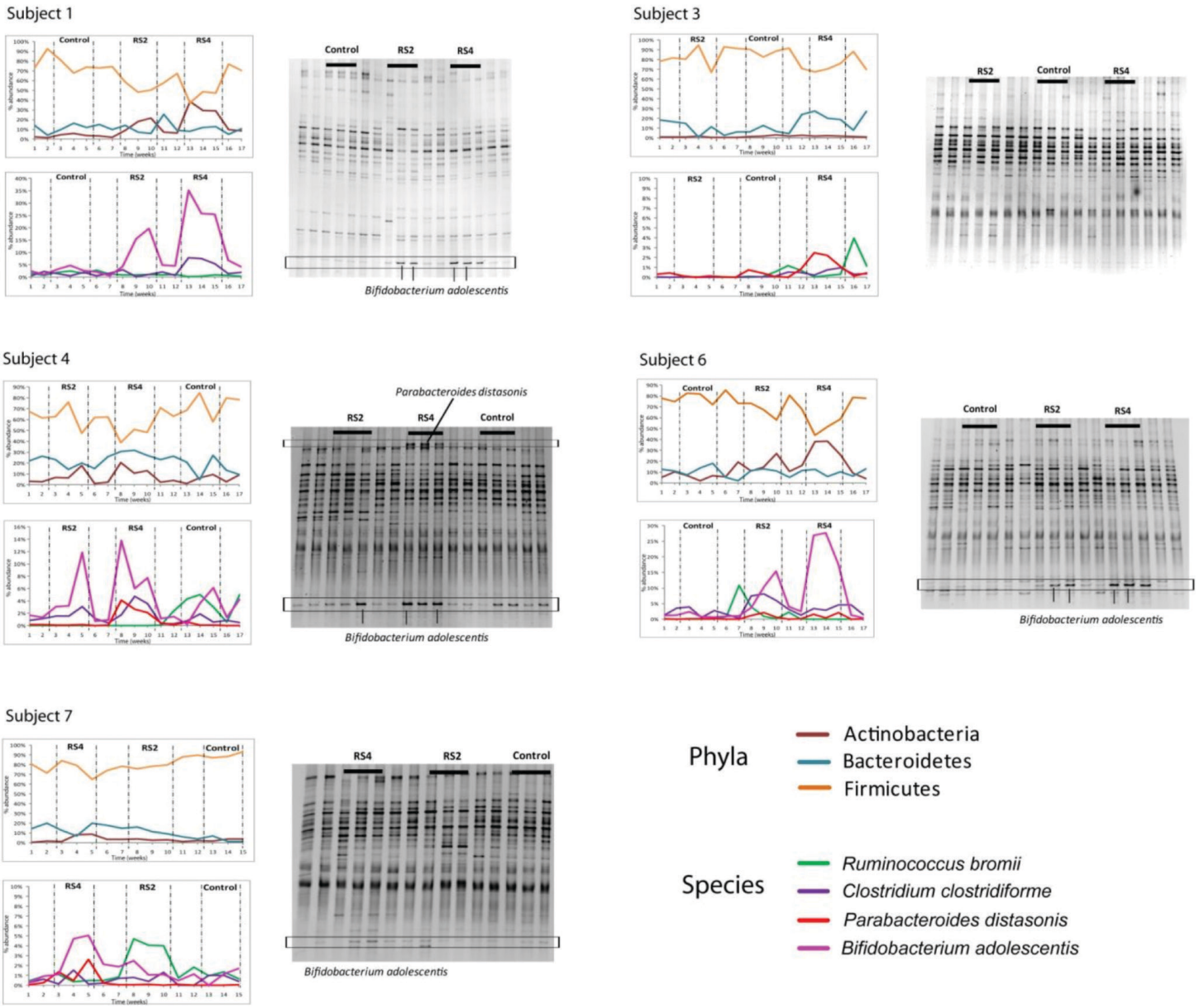
[3]*The bacteria populations are averages of all the six time points of the three washout periods.*

[4]*Bacterial populations during the dietary treatments were compared to each other with repeated measures ANOVA and Tukey's post hoc test. Numbers in bold represent proportions that were significantly higher than the numbers shown in italic.*

relates to weight loss. *Bifidobacteria* are known to be strongly correlated with immunological and metabolic improvements of Type 2 diabetes and high blood cholesterol levels in animal models. Furthermore, Fibersym® reduced the level of Erysipelotrichaceae (**Table 7**), a bacterial family that includes members that have been shown to be positively associated with an increase in liver fat in women. At the species level, Fibersym® increased the populations of *Bifidobacterium adolescentis* as stated earlier and also *Parabacteroides distasonis* (**Fig. 3**).

Weekly symptom diaries documented by the subjects revealed no significant detrimental effects on bowel movement, stool consistency, or discomfort. Only a

Figure 3. *Temporal dynamics of the human fecal microbiota in response to the consumption of crackers containing native (control) and resistant starches in five human subjects. RS2 is RS2 high-amylose maize starch; RS4 is Fibersym® RW; Control is native wheat starch.*



Reproduced with permission from *Martinez, I., Kim, J., Duffy, P.R., Schlegel, V.L., and Walter, J. 2010. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. PLoS ONE 5(11):e15046.doi:10.1371/journal.pone.0015046*

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moderate increase in flatulence was observed (**Table 8**). This indicates that Fibersym® RW at a dose of 33 grams per day is well tolerated by human subjects.

The butyrogenic effect of Fibersym® can be easily demonstrated by in vitro fermentation rather than by in vivo experiments because butyric acid is absorbed by the epithelial cells lining the colon of a subject. In a study conducted at the University of Toronto, Fibersym® was cooked in aqueous media and then sequentially digested in vitro by pepsin (to simulate stomach digestion) and pancreatin-bile (to simulate small intestinal digestion and absorption). The indigestible residue was subsequently fermented after being inoculated with fresh human fecal microbiota (to simulate large bowel fermentation). The results demonstrated that 82% of Fibersym® was recovered as indigestible residue, which upon fermentation produced gas that increased linearly over the 24-hour fermentation period (**Table 9**) and short chain fatty acids that increased during the 24-hour period, but tended to level off after 8 hours (**Table 10**).

Acetate was the major short chain fatty acid followed by butyrate and propionate, with a corresponding molar ratio of 59:23:19. These short chain fatty acids have been known to be the major energy source of colon cells,

Table 8. *Mean ± standard deviations of weekly symptoms reported by the subjects on a scale from 1 (best) to 5 (worse). RS2 is RS2 high-amylose maize starch: RS4 is Fibersym® RW: Control is native wheat starch.*

	RS2	RS4	Control	None
Bowel movement	1.73 ± 0.83	1.90 ± 0.93	1.73 ± 0.77	1.74 ± 0.59
Stool consistency	2.07 ± 1.29	2.23 ± 0.92	2.03 ± 0.91	2.00 ± 0.83
Discomfort	1.65 ± 0.65	1.87 ± 0.79	1.50 ± 0.55	1.53 ± 0.40
Flatulence*	2.42 ± 1.28	2.27 ± 1.00	1.37 ± 0.58	1.54 ± 0.43
Abdominal pain	1.63 ± 0.79	1.47 ± 0.67	1.40 ± 0.60	1.36 ± 0.62
Bloating	1.67 ± 0.98	1.40 ± 0.52	1.07 ± 0.14	1.29 ± 0.45

* Significant differences were detected by ANOVA (P<0.05). Tukey's post-hoc test did not detect significance in pair-wise comparisons.

Reproduced with permission from *Martinez, I., Kim, J., Duffy, P.R., Schlegel, V.L., and Walter, J. 2010. Resistant starches type 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. PLoS ONE 5(11):e15046.doi:10.1371/journal.pone.0015046.*

Table 9. *Cumulative total gas production (ml/g of starch) at various times upon fermentation of indigestible residues obtained from Fibersym® RW.*

	FERMENTATION TIME (HR)			
Sample	**4**	**8**	**12**	**24**
Fibersym® RW	*78.0 ± 0a*	*118.5 ± 5.8b*	*153.3 ± 5.8c*	*214.2 ± 2.8d*

Adapted with permission from *Thompson, L.U., Maningat, C.C., Woo, K., and Seib, P.A. 2011. In vitro digestion of RS4-type resistant wheat and potato starches and fermentation of indigestible fractions. Cereal Chem. 88:72-79.*

Table 10. *Cumulative short chain fatty acid (SCFA) production (mmol/g of starch) at various times of indigestible residues obtained from Fibersym® RW[a].*

	FERMENTATION TIME (HR)			
SCFA[b,c]	4	8	12	24
C1	0.50 ± 1.08ab	0.69 ± 0.10ab	0.76 ± 0.15a	0.00 ± 0.00b
C2	1.85 ± 0.35a	2.87 ± 0.27ab	3.44 ± 0.03ab	3.85 ± 0.21b
C3	0.39 ± 0.02a	1.05 ± 0.03b	1.10 ± 0.04b	1.22 ± 0.04b
iC4	0.01 ± 0.01a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
C4	0.40 ± 0.10a	0.93 ± 0.08b	1.32 ± 0.00bc	1.50 ± 0.05c
iC5	0.04 ± 0.03a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
C5	0.05 ± 0.03a	0.04 ± 0.00a	0.04 ± 0.01a	0.01 ± 0.01a
Total	3.25 ± 0.60a	5.58 ± 0.48ab	6.66 ± 0.21b	6.58 ± 0.30b

[a] *Mean ± SEM; Mean values (± SEM) in the same row followed by different letters are significantly different (P < 0.05).*

[b] *SCFA, short chain fatty acids; C1, formic acid; C2, acetic acid; C3, propionic acid; iC4, isobutyric acid; C4, butyric acid; iC5, isovaleric acid; C5, valeric acid.*

[c] *Fractional molar ratio of C2:C3:C4 at 24 hr for Fibersym® RW = 0.586:0.186:0.228.*

Adapted with permission from *Thompson, L.U., Maningat, C.C., Woo, K., Seib, P.A. 2011. In vitro digestion of RS4-type resistant wheat and potato starches, and fermentation of indigestible fractions. Cereal Chem. 88:72-79.*

and tend to lower colonic pH, allowing the favorable growth of beneficial microorganisms. In particular, the increase in butyrate, which is the preferred substrate by colon cells, may be protective against colon cancer as it is known to decrease cancer cell proliferation and increase apoptosis (programmed cell death) and cell differentiation.

Microscopic examination of the indigestible residue of Fibersym® recovered after its digestion with pepsin and pancreatin-bile revealed that the pattern of digestion is by surface erosion (Fig. 4).

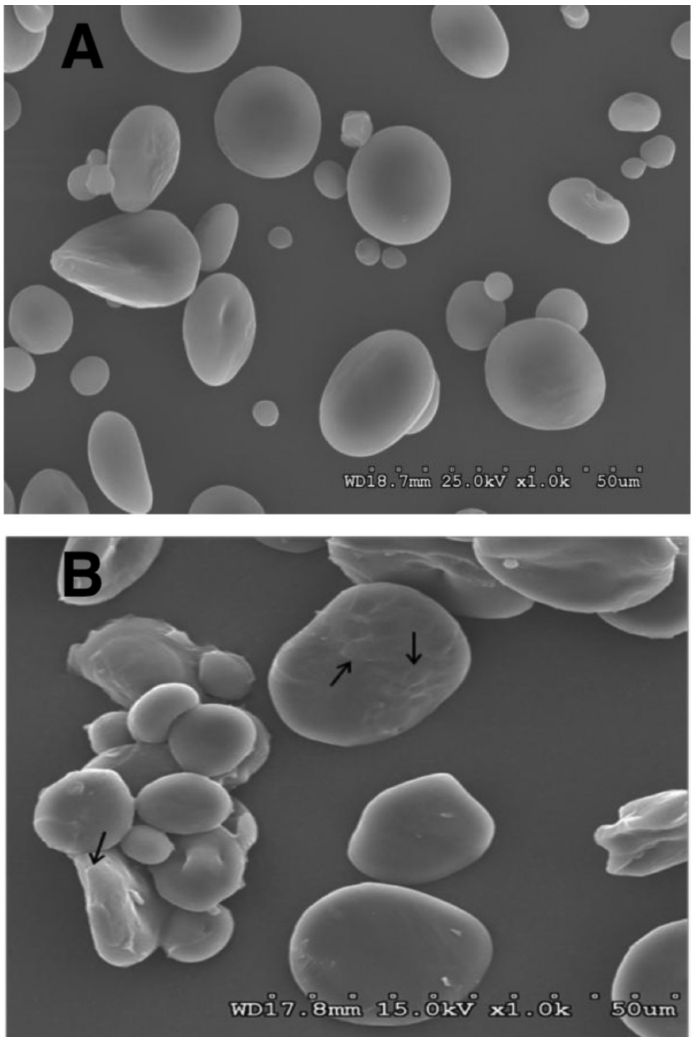


FIGURE 4. *Scanning electron micrographs of Fibersym® RW (A) and indigestible residues after cooking in water (boiling water bath) followed by in vitro digestion with pepsin and pancreatin-bile (B). Arrows indicate areas with surface erosion or pieces peeling off.*
Reproduced with permission from *Thompson, L.U., Maningat, C.C., Woo, K., and Seib, P.A. 2011. In vitro digestion of RS4-type resistant wheat and potato starches, and fermentation of indigestible fractions. Cereal Chem. 88:72-79.*

IMPACT ON GASTROINTESTINAL HEALTH ▸ *Continued*

This pattern of enzyme attack by mammalian amylase concurs with that observed on indigestible residues of Fibersym® remaining after microbial amylase digestion in AOAC Method 991.43 for the determination of total dietary fiber (**Fig. 5**).

Fibersym® increases colonic fermentation/short-chain fatty acid production and contributes to positive modulation of colonic microflora. These physiological effects were demonstrated in human studies conducted at South Dakota State University and the University of Nebraska.

In the South Dakota State University study, the individual proportions of short chain fatty acids, namely butyric (69.5%, p=0.03), propionic (50.2%), valeric (44.1%), isovaleric (20.3%) and hexanoic (19.2%) acids increased

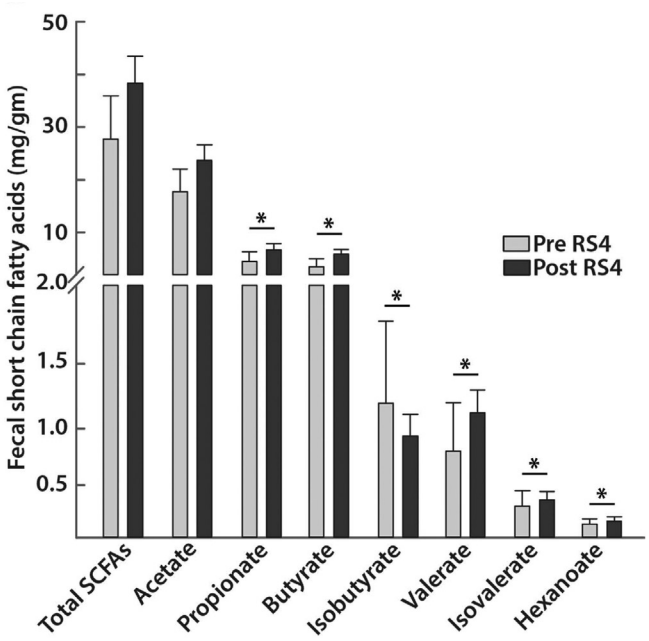


Figure 6. *Abundance of short-chain fatty acids (SCFA) before (grey bar) and after (black bar) Fibersym-Flour blend intervention (*p≤0.05, n=19).*

Reproduced with permission from *Upadhyaya, B., McCormack, L., Fardin-Kia, A.R., Juenemann, R., Nichenametla, S., Clapper, J., Specker, B., and Dey, M. 2016. Impact of dietary resistant starch type 4 on human gut microbiota and immunometabolic functions. Scientific Reports 6:28797; doi:10.1038/srep28797.* ***http://creativecommons.org/licenses/by/4.0/***

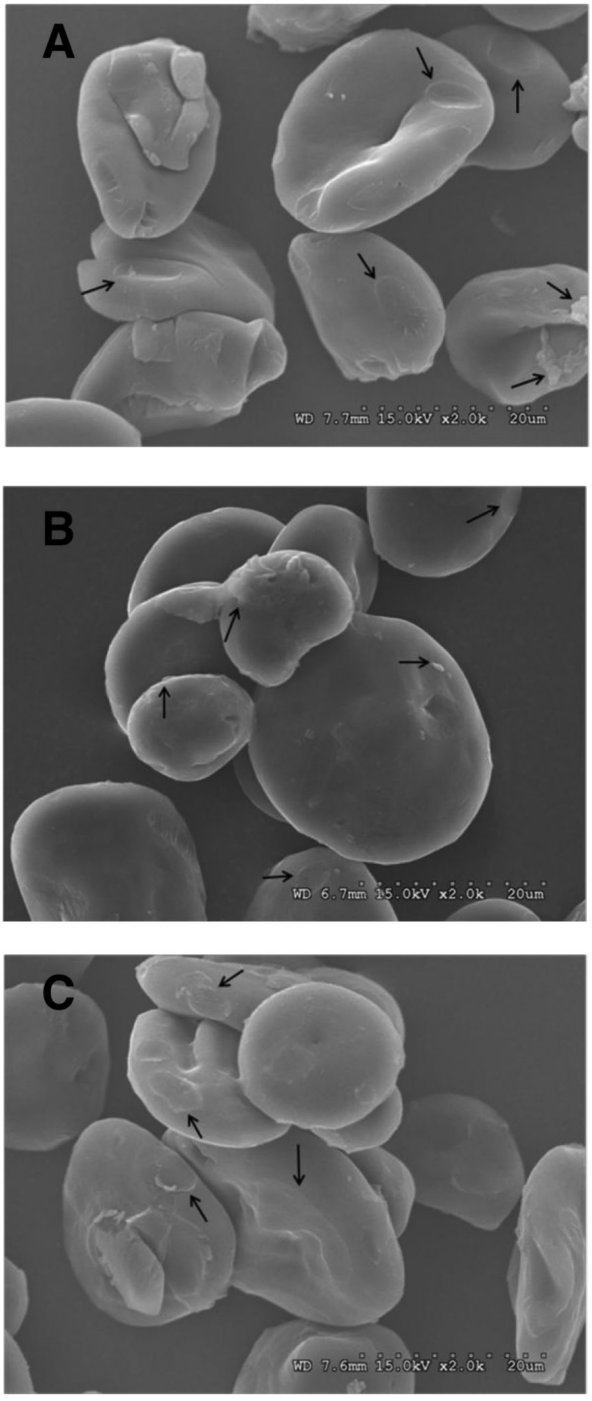


Figure 5. *Appearance of Fibersym® RW after hydrolysis with alpha-amylase (A); after hydrolysis with alpha-amylase and protease (B); and after hydrolysis with alpha-amylase, protease, and glucoamylase (C) according to total dietary fiber determination by AOAC Method 991.43. Arrows indicate areas with surface erosion or pieces peeling off.*

Reproduced with permission from *Thompson, L.U., Maningat, C.C., Woo, K., and Seib, P.A. 2011. In vitro digestion of RS4-type resistant wheat and potato starches, and fermentation of indigestible fractions. Cereal Chem. 88:72-79.*

post intervention from baseline in the Fibersym-Flour group ($p<0.05$, Fig. 6) but not in the Control Flour group. A potential link exists between significant changes in the gut microbiota composition induced by Fibersym® and the altered levels of short chain fatty acids. *Christensenella minuta* was enriched post Fibersym-Flour diet compared with post-Control Flour diet and the baseline, and correlated in a Fibersym-specific manner with higher propionate, isobutyrate, valerate and isovalerate concentrations (Fig. 7). Acetate and butyrate levels were correlated ($p<0.05$), respectively, with *Ruminococcus lactaris* ($r=0.54$) and *Oscillospira* spp. ($r=0.41$). Total short chain fatty acids were correlated with the abundance of *Methanobrevibacter* spp. ($r=0.43$) and *Ruminococcus lactaris* ($r=0.52$). Propionate and isobutyrate levels were linked to *Methanobrevibacter* spp. ($r=0.65$ and $r=0.79$, respectively), *Eubacterium dolichum* ($r=0.42$ and $r=0.43$, respectively), *Christensenella minuta* ($r=0.39$ and $r=0.59$, respectively), and *Ruminococcus lactaris* ($r=0.59$ and $r=0.40$, respectively), of which the latter two were increased by Fibersym. These associations of short chain fatty acids with specific gut microbiota were not observed after Control Flour intervention.

At the species level, some species were significantly enriched in the Fibersym-Flour blend group relative to the Control Flour group including three *Bacteroides* species along with *Blautia glucerasea*, *Christensenella minuta*, *Eubacterium oxidoreducens*, *Oscillospira* spp., *Ruminococcus lactaris*, and *Parabacteroides distasonis* (Fig. 8). Some species were significantly decreased in abundance in this group, including the pathogenic *Enterococcus casseliflavus* and *Streptococcus cristatus*.

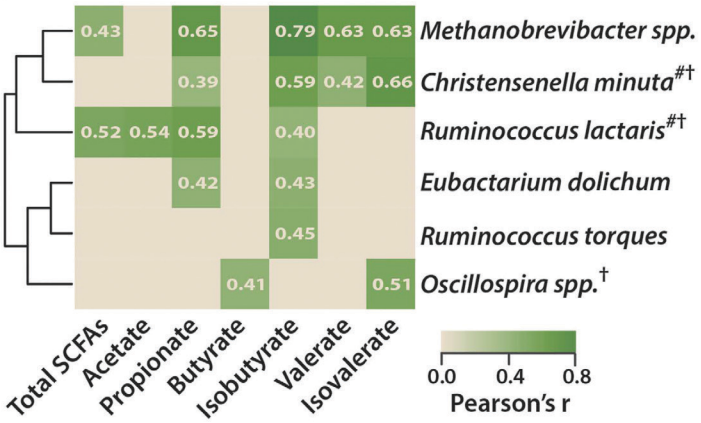


Figure 7. *Positive correlation of six bacterial species with increased short-chain fatty acid levels in a Fibersym-specific manner (all, $p<0.05$). Pearson coefficients are shown on heat map. #, the closest hit from the NCBI 16S rRNA database cross-referenced with the OTU from the Greengenes database. †, species either significantly enriched or approached significance in the Fibersym®group.*

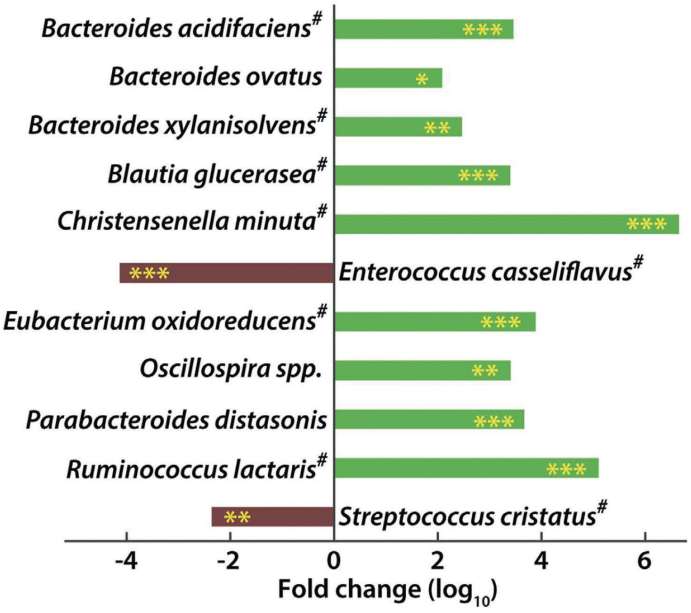


Figure 8. *Relative abundance of bacterial species (log fold change) in the Fibersym-Flour blend group compared with Control Flour group post intervention (n=19).*

After Fibersym® intervention (compared to the baseline), Fibersym-Flour blend consumption increased the abundance of *Bifidobacterium adolescentis* and *Parabacteroidetes distasonis*, but not *Ruminococcus bromii*, *Faecalibacterium prausnutzii* or *Dorea formicigenerans* (Fig. 9). A novel observation was a Fibersym-induced increase in *Christensenella minuta* abundance as well as in the family *Ruminococcaceae* and genus *Bacteroides*. At the species level, Bacteroides ovatus, *Ruminococcus lactaris*, *Eubacterium oxidoreducens*, *Bacteroides xylanisolvens*, and *Bacteroides acidifaciens* were enriched after Fibersym® intervention.

IMPACT ON GASTROINTESTINAL HEALTH ▸ *Continued*

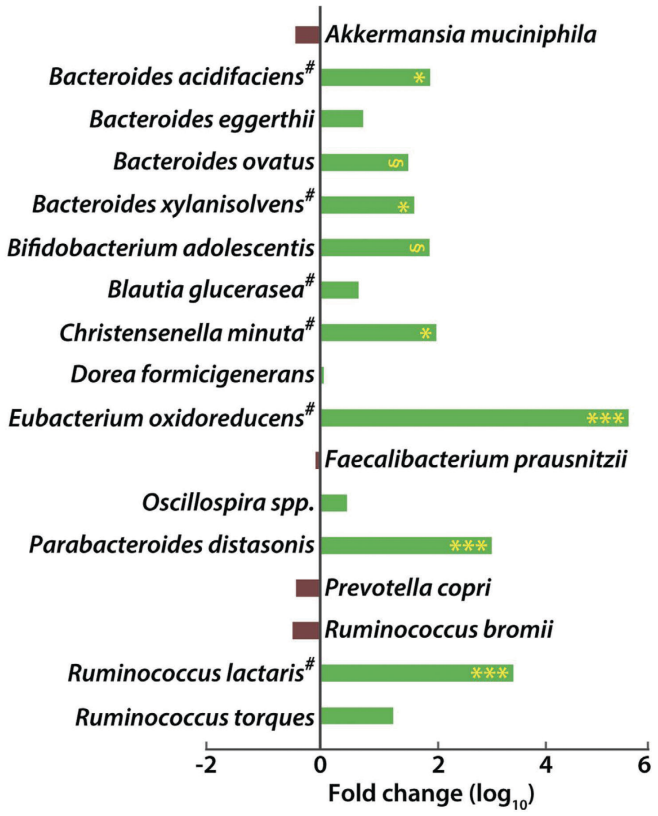


Figure 9. *Abundance of major bacterial species (log fold change) before and after Fibersym-Flour blend treatment. #, the closest hit from the NCBI 16S rRNA database cross-referenced with the OTU from the Greengenes database. *q≤0.05, **q≤0.01, ***q≤0.001, §q≤0.09 (trend/approaching significance), n=19.*

In the University of Nebraska study already mentioned above, additional observations with statistical analysis data indicate that Fibersym® consumption resulted in significant decreases in Firmicutes ($p<0.001$) by more than 10% on average, and increases in Bacteroidetes ($p<0.01$) and Actinobacteria ($p<0.05$) by around 5% each. These changes were associated with a decrease in the family Ruminococcaceae ($p<0.01$) and increases in the genera *Parabacteroides* ($p<0.001$) and *Bifidobacterium* ($p<0.05$). The species responsible for the resistant starch-induced changes in the genera Bifidobacterium, Parabacteroides, Faecalibacterium and Dorea corresponded to *Bifidobacterium adolescentis* ($p < 0.05$), *Parabacteroides distasonis* ($p<0.001$), *Faecalibacterium praustnitzii* ($p<0.05$) and *Dorea formicigenerans* ($p<0.05$), respectively. The proportion of *Clostridium clostridioforme* was increased by both Fibersym® and Hi-Maize 260, and the increase reached statistical significance for Fibersym® ($p<0.05$). Furthermore, the abundance of the species Eubacterium rectale ($p<0.05$) and Ruminococcus bromii ($p<0.05$) were significantly increased when Hi-Maize 260 was consumed when compared to Fibersym®. This study demonstrated that resistant starch promoted distinct compositional alterations within the human gut microbiota. Specific bacterial populations can be selectively targeted by resistant starch.

As also shown in the South Dakota State University study, *Parabacteroides distasonis* was augmented post Fibersym-Flour diet and this species showed a negative correlation with total cholesterol ($r=-0.52$), LDL cholesterol ($r=-0.50$) and non-HDL cholesterol ($r=-0.52$) in both intervention groups (**Fig. 10**).

A Fibersym-specific correlation between adiponectin and *Bacteroides acidifaciens* ($r=0.82$, $p<0.001$) was observed. *Methanobrevibacter* spp. and *Eubacterium dolichum* were correlated with weight and body mass index as well as with short chain fatty acid levels in a Fibersym-specific manner. *Methanobrevibacter* spp. ($r=-0.45$), *Ruminococcus gnavus* ($r=-0.56$) and *Prevotella stercorea* ($r=-0.45$) were negatively correlated with LDL cholesterol ($p<0.05$), while *Blautia producta* ($r=-0.44$) and *Prevotella stercorea* ($r=-0.50$) were negatively associated with total cholesterol and non-HDL cholesterol (all, $p<0.05$) in a Fibersym-specific manner. This study provides evidence that dietary Fibersym® supplementation selectively changes the gut microbial and metabolite environment as well as associated host metabolic functions.

The concept of colonic health led to the development of functional foods such as probiotics, prebiotics and synbiotics and other dietary components that target the colon and affect its environment, composition of microflora, and physiology of the colon, and display distinct health benefits. Gut microbiota have been linked to reduction of obesity and the risk of colon cancer.

Obesity is closely tied to phylum and group-specific changes in human gut microbiota. A significant reduction of *Bacteroidetes* and a corresponding increase in *Firmicutes* is observed within individual humans on weight reduction diets. As discussed above, Fibersym® decreases the ratio of *Firmicutes/Bacteroidetes* in humans after a 3-week feeding study. The decrease in *Firmicutes/Bacteroidetes* ratio was also demonstrated in the second South Dakota State University study (**Fig. 11**).

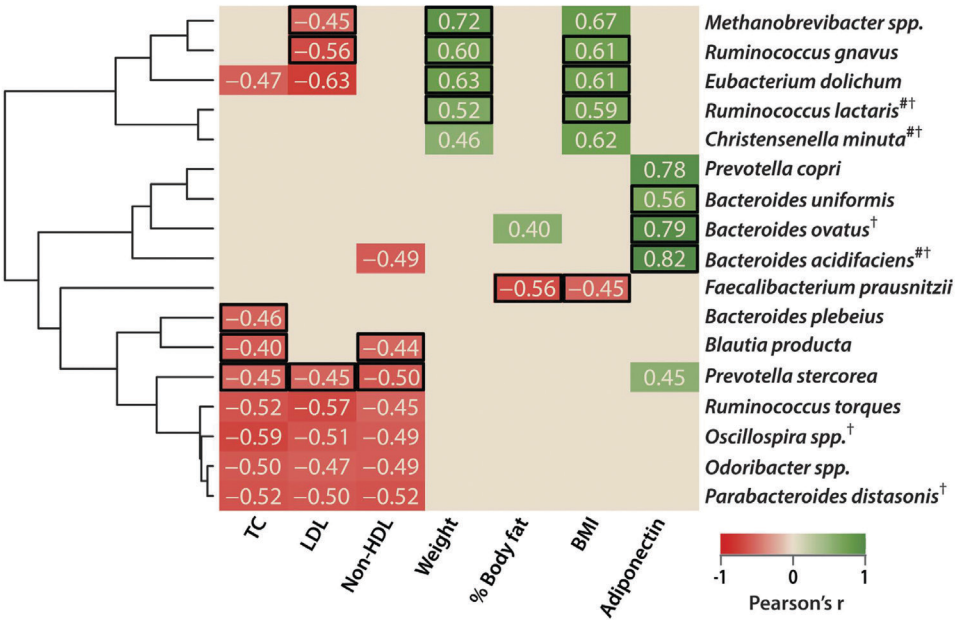


Figure 10. *Association between gut microbiota and host biological parameters after Control Flour and Fibersym-Flour blend interventions. Heat map showing Pearson's r values (all, p<0.05). Black rectangular borders indicate an association present only post Fibersym-Flour blend intervention. #, the closest hit from the NCBI 16S rRNA database cross-referenced with the OTU from the Greengenes database. †, species either significantly enriched or approached significance in the Fibersym-Flour blend group, n=15.*

These alterations in relative proportions of *Firmicutes* and *Bacteroidetes* were correlated with observations in lean and obese humans, and suggest that the microbiota of an obese person is more efficient at extracting energy from the diet than that of a lean individual. Other studies also found higher *Firmicutes/Bacteroidetes* ratio in obese individuals compared to lean individuals demonstrating that obesity is associated with reduced bacterial representation of *Bacteroidetes*. Other feeding studies over a 10-week period on overweight or obese adolescents demonstrated an increase in *Bacteroides* group in those adolescents that experienced weight loss. Clearly, modulation of gut microbiota could be a useful and alternative approach for weight reduction or for the treatment of obese patients.

The increased population of Bacteroides species in the Fibersym-flour blend group led to an overall lowering of the average Firmicutes/Bacteroidetes ratio in the Fibersym-flour blend group from 14.6 at baseline to 12.9, but increasing to 19.2 with the Control flour group (**Fig. 11**).

Colonic fermentation of dietary fiber/resistant starch produces short chain fatty acids such as acetate, propionate and butyrate. Gut microflora-associated short chain fatty acids are particularly useful biomarkers for cancer. Histone deacetylase inhibitors have been widely used for cancer therapy. Butyrate, the primary energy source for colonocytes, and, to a lesser extent, propionate are known to act as histone deacetylase inhibitors thereby conferring short-chain fatty acids the role of modulators of cancer. Moreover, butyrate seems to have a protective role based on a significant decrease in the number of butyrate-producing bacteria in the colon of patients with ulcerative colitis and colon cancer. Certain prebiotics reduce fecal water genotoxicity in humans resulting in reduced colon cancer risk. An intervention study with dietary fiber mixed with probiotics in human subjects favorably altered colon cancer biomarkers in high-risk individuals.

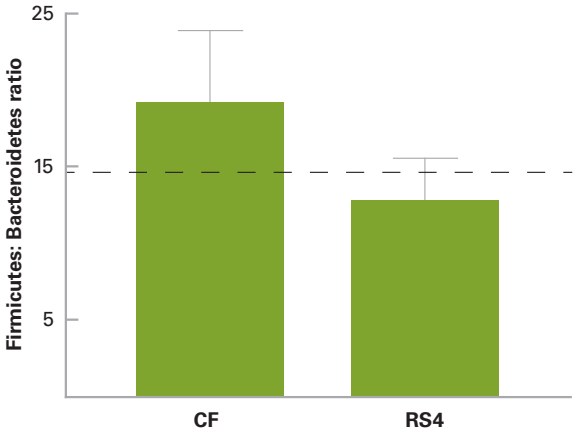


Figure 11. *The Firmicutes/Bacteroidetes ratio after Control Flour (CF) and Fibersym-Flour blend (RS4) intervention (n=14). The dotted line represents this ratio at baseline.*

ROLE IN WEIGHT MANAGEMENT

One of the most visible, but challenging, medical conditions to treat is obesity, which is defined as a body mass index exceeding 30. Body mass index, or BMI, is a rough estimate of a healthy body weight based on the height of an adult individual. It is calculated by dividing a person's weight in kilograms by the square of his or her height in meters. A BMI of 18.5-24.9 is considered normal weight, a range of 25-29.9 is considered overweight, and less than 18.5 is underweight. It is estimated that an average American male has gained 17.1 pounds since 1998, while the average female has gained 15.4 pounds.

The statistics on obesity are disturbing. A 2018 report from Trust for America's Health and the Robert Wood Johnson Foundation found that obesity continues to be a significant health problem throughout the U.S. According to the most recent National Health and Nutrition Examination Survey (NHANES), 18.5% of children and 39.6% of adults were considered obese in 2015-2016. In a CNN report, overweight children have been labeled "coronary time bombs," as they are likely to develop heart disease when they grow into adulthood. States in the Northeast and West had lower overall obesity rates than those in the South and Midwest. West Virginia topped the list as the most obese state in the nation, while Colorado is the least obese state. West Virginia was also ranked to have the highest adult diabetes and hypertension rates and Utah had the lowest.

Obesity costs the country billions of dollars in weight-related bills. The high price tag is simply because obesity is not a singular medical condition. Being overweight or obese is also associated with Type 2 diabetes, heart disease, high cholesterol, hypertension, osteoarthritis, stroke, sleep apnea, asthma, cancer, and kidney and gall bladder disease. Obesity is responsible for about 100,000 cancer cases each year. Such alarming information about obesity and its toll on the human body has led to myriads of studies to deter its occurrence.

How is fiber or resistant starch related to weight management? Evidence exists based on food consumption data and clinical studies regarding the inverse relationship between fiber and obesity. Those who ingested the most dietary fiber had lower mean energy intakes and lower BMI values, while those ingesting the least fiber tended to be the most obese.

Body weight is a reflection of the balance between two variables: the calories a body takes in and the calories it burns off. It is proposed that fiber acts as a physiological obstacle to energy intake by several mechanisms, which helps explain the role of fiber in weight management. Diets with adequate fiber are generally less energy dense (low caloric count). Furthermore, diets with sufficient fiber occupy greater food volumes (or bulk), which may curb intake of other foods. When ingested, the large food volume stays in the stomach longer, resulting in an increased sensation of fullness. Fiber or resistant starch reduces glycemic response and promotes satiety, which is a feeling that comes after eating a meal and inhibits a person from eating again within a relatively short time span.

Fibersym® has a low caloric count (~90% lower than native wheat starch), which demonstrates its usefulness in reduced-calorie foods. Native wheat starch (Midsol 50) has a caloric count of 356 kcal/100 g or ~3.6 kcal/g. Fibersym® has a typical nutritional composition of 0.15% protein, 0.5% total fat, 1.25% ash, 79.6% insoluble fiber, 1% soluble fiber and 10.5% moisture. Using the calorie calculation published by FDA in the Federal Register in 2016, the caloric count of Fibersym® is 35.1 kcal/100 g or ~0.4 kcal/g.

In the preamble to the 2016 Nutrition Facts Rule, FDA identified a number of physiological effects that it considers to be beneficial to human health, including increased satiety associated with reduced energy intake, which can reduce the risk of being overweight or obese. FDA also clarified that the effects identified in the preamble do not constitute an exhaustive list of effects that may be beneficial to human health. As described in more detail below, two human studies have demonstrated that consumption of Fibersym® significantly reduces both body fat percentage and waist circumference, both of which reduce the risk of being overweight or obese. While these studies did not directly measure satiety, they clearly indicate that consumption of Fibersym® provides a benefit by reducing the risk of being overweight or obese, just as any fiber ingredient that increases satiety does (and through a measure that is more directly linked to weight loss than satiety). Therefore, the outcomes identified in these studies demonstrate a beneficial physiological effect: reduced body fat and waist circumference, indicating a reduced risk of being overweight or obese.

In the first South Dakota State University study previously discussed under the Section titled Role in Cholesterol Reduction, healthy individuals without metabolic syndrome had a 2.6% smaller ($p=0.02$) waist circumference and 1.5% lower ($p=0.03$) percent body fat following Fibersym-Flour intervention compared to Control Flour. A small but significant 1% increase in fat-

free mass was also observed in all participants combined (p=0.02). No significant gastrointestinal side effects were observed such as constipation (p=0.62), diarrhea (p=0.18), bloating/gas (p=0.10) and abdominal pain (p=0.31).

In a follow-up study at South Dakota State University involving 20 healthy individuals with metabolic syndrome (12 females and eight males, aged 32-77), the individuals had lower % body fat (p=0.05) and trended towards lower waist circumference (p=0.06) following Fibersym-Flour consumption compared with Control Flour consumption. Waist circumference was also reduced in the Fibersym-Flour group compared with baseline (p<0.05). Attenuation of % body fat combined with a smaller waist circumference indicates a potential reduction in central obesity in these individuals.

PHYSIOLOGICAL EFFECTS FROM ANIMAL STUDIES

In an animal feeding trial conducted at Kansas State University, growing hamsters were fed a diet containing 15% protein, 9.9% fat, 0.5% cholesterol, and 10% of dietary fiber either from cellulose or phosphorylated cross-linked (P-CL) wheat starch, which is exactly the same RS4 as Fibersym). The remainder of each diet was mainly corn starch with low levels of minerals, vitamins and choline. The sample of P-CL wheat starch (RS4) was prepared in the laboratory and contained 76% total dietary fiber determined by the AOAC Method 991.43. During the 6 weeks of feeding, the animals on the RS4 diet consumed less feed than those on the cellulose diet, and after six weeks they weighed ~10% less than the cellulose group. Total serum cholesterol was the same (261 vs 265 mg/dL) in both groups, but the high-density lipoprotein fraction (HDL), which is the so-called good cholesterol, was elevated 15% (139 vs 160 mg/dL) in the RS4 group, whereas the bad cholesterol was lowered by 21% (122 vs 96 mg/dL). The bad cholesterol is the sum of cholesterol in the low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) fractions. The animals consuming RS4 diet showed a 22% increase in short-chain fatty acids in the contents of their cecum (19.8 vs 30.3 mmol/g of wet cecum), which included an almost 3000% increase in butyric acid (0.1 vs 2.7 mmol/g). This study indicates that Fibersym®can increase good cholesterol and decrease bad cholesterol, which favorably impacts cardiovascular health. Also, the study supports the idea that Fibersym®is good for gut health because it is fermented in the colon to produce short-chain fatty acids, especially butyric acid. Finally, the animals ate fewer calories when the feed contained P-CL wheat starch. That result could be because P-CL wheat starch induced satiety in the animal model or because of decreased palatability.

Scientists at South Dakota State University investigated the *in vivo* effects of Fibersym®on butyrate production in the cecum and butyrate-associated regulation of inflammatory markers of colon tissues in mice. Six-week old male mice were grouped randomly to consume either a Fibersym®diet or a control diet, both diets being isocaloric. Mice were caged in groups of three at 24-26°C and 12 hours of light/dark cycle. During the first 3 weeks, mice were acclimatized on a standard rodent chow diet. Animals were then switched to experimental diets. Mice were provided with experimental diets and water *ad libitum* for 12 weeks. At the conclusion of the 12-week feeding period, cumulative food intake in the Fibersym®group was around 35% higher compared to the Control group. However, cumulative body weight increase in both groups was similar during the 12-week period. The cecal weight of mice on the Fibersym®diet was higher compared to mice on the Control diet which is typical for diets differing in levels of dietary fiber. Butyrate concentration of cecal samples was twice as high in the Fibersym®group than in the control group. Analysis of mouse colon tissues, as well as colon cells of human origin, revealed that Fibersym®and butyrate may function as an epigenetic repressor of pro-inflammatory mediators.

Gut microbiota influences human health and it stands to reason that dietary factors which affect species composition and metabolic characteristics have

PHYSIOLOGICAL EFFECTS FROM ANIMAL STUDIES ▸ *Continued*

a role in disease prevention. Resistant starch improves insulin sensitivity in clinical trials, but the mechanisms underlying this health benefit remain inadequately understood. The gut microbiota is generally assumed to influence associated health benefits by fermenting resistant starch to short-chain fatty acids, which have numerous metabolic effects on host physiology. The role of gut microbiota in mediating the metabolic benefits of resistant starch especially as it relates to improving insulin sensitivity was determined by University of Nebraska scientists using mice. The researchers compared the effects of feeding RS2 Hi-Maize 260 and RS4 Fibersym® in Western diet-fed mice with or without microbiota to determine their impact on insulin sensitivity. Germ-free male mice maintained at gnotobiotic conditions and conventionalized mice were used in the study. Experimental diets were introduced to both mice and body weight and food intake were monitored weekly. This study unequivocally demonstrated that some metabolic benefits exerted by dietary resistant starch, especially improvements in insulin levels, occur independently of the microbiota. Feeding Hi-Maize 260 and Fibersym® resulted in vastly different effects on the composition of gut microbiota. However, both RS types induced physiological changes that were highly similar, with RS4 Fibersym® more consistently improving metabolic parameters as compared to RS2 Hi-Maize 260. The researchers proposed a model in which resistant starch induces health benefits via both microbiota-dependent and microbiota-independent pathways. When resistant starch is fed at higher doses, metabolites from bacterial fermentation are released at physiologically relevant levels, leading to a decrease in body weight and/or fat mass. When fed in clinically relevant doses, resistant starch improves insulin resistance independently of the gut microbiota, possibly altering bile acid signaling as well as adipose tissue immune modulation.

FUNCTIONAL PERFORMANCE OF FIBERSYM® RW IN FOODS

Product developers add Fibersym® in food products to boost fiber content for nutrient labeling claims and to lower caloric count. Two nutrient claims can be applied to food products with respect to the level of dietary fiber per serving size. According to 21 CFR 101.54, “high” or “excellent source of” fiber may be used on the label and in the labeling of foods provided that the food contains 20% or more of the Recommended Daily Intake (RDI) or the Daily Recommended Value (DRV) per reference amount customarily consumed (RACC). The RACC that applies to a food is the unit amount of a food that must be used by companies to determine the amount of food that constitutes a “serving”. The terms “good source of” fiber may be used on the label or in the labeling of foods provided that the food contains 10 to 19% of the RDI or the DRV per RACC. According to 21 CFR 101.60, the terms “reduced calorie” or “lower calorie” may be used on the label or in the labeling of foods provided that the food contains at least 25% fewer calories per RACC than an appropriate reference food. As stated in 21 CFR 101.9, the caloric content of foods may be calculated using the general factors of 4, 4, and 9 calories per gram for protein, total carbohydrate (less the amount of non-digestible carbohydrates and sugar alcohols), and total fat, respectively, as described in USDA Handbook No. 74 (slightly revised, 1973) pages 9-11. A general factor of 2 calories per gram for soluble non-digestible carbohydrate shall be used. The general factors for caloric value of the different sugar alcohols are provided in 21 CFR 101.9. It should be noted that serving size differs among consumer food products. FDA defined serving sizes of food products in 21 CFR 101.12.

Food product formulators can take advantage of the following key points of Fibersym® over other resistant starches as well as conventional dietary fiber sources. First, Fibersym® has the highest level of total dietary fiber (90% minimum, dry basis) versus other resistant starches. Because of less usage levels in food formulations, end-users can realize cost savings. Second, Fibersym® is white in color, making it an ideal source of “invisible” fiber. For example, it does not detract from the appearance of bakery products, which makes it perfectly suitable for white bread and cake applications. Third, its microscopic smooth-surfaced particles provide a non-gritty texture in food products versus the coarse granulation and gritty particles of cereal brans. Fourth, Fibersym® absorbs approximately the same amount of water as wheat flour, meaning little or no formulation or processing changes are required to produce wheat-based foods, such as bakery products, pasta, and noodles. The measured water holding capacity is 0.7 g of water per gram of Fibersym. Often, little or no change is required in water absorption, mixing time, and baking time of dough products, probably because the wheat starch present in flour, which accounts for about three-fourths its weight, has the same composition and size, shape and surface properties compared to the wheat starch-base in Fibersym. Fifth, Fibersym® has low caloric content. Its calculated caloric count is ~0.4 kcal/g, which is about 10% of that in native wheat starch at ~3.6 kcal/g.

Table 11. *Dough and bread characteristics of control and high-protein, high-fiber (HPHF) formulas.*

PROPERTY	WHITE FLOUR		WHOLE WHEAT RED FLOUR		WHOLE WHEAT WHITE FLOUR	
	Control	HPHF	Control	HPHF	Control	HPHF
Absorption, %	63	77	69.7	78	75.7	80
Mixing Time, min.	10	5	8.5	5	8	5
Proof Time, min.	68	45	53	36	52	34
Bread volume, cc	2441	2766	2200	2460	2144	2416
Specific volume, cc/g	5.18	6.06	4.63	5.32	4.52	5.21
Total Quality Score	83.8	79.4	86.5	80.7	80.2	78.5
Moisture, g/100g	35.9	39.8	35.3	39.9	36.4	39.6
Protein, g/100g	8.6	17.6	11.6	17.8	10.8	17.6
Dietary fiber, g/100g	2.0	17.7	6.4	19.0	6.8	19.2
Calories, kcal/100g	258	185	245	179	241	180

Adapted from *Maningat, C., Bassi, S., Woo, K., Dohl, C., Gaul, J., Stempien, G., and Moore, T. 2005. Formulation of high-protein, high-fiber (low carbohydrate), reduced calorie breads. AIB Tech. Bull. 27(4):1-16.* Used with permission.

PROVEN BENEFITS OF FIBERSYM® RW AS A DIETARY FIBER SOURCE

HIGH-PROTEIN, HIGH-FIBER BREADS

In a study conducted at the American Institute of Baking International (Manhattan, KS), high-protein, high-fiber (HPHF) white or whole wheat bread doughs formulated with 11.6% Fibersym® (based on total formula weight) had higher water absorption and less mixing time (3 to 5 minutes shorter) than the control doughs (**Table 11**).

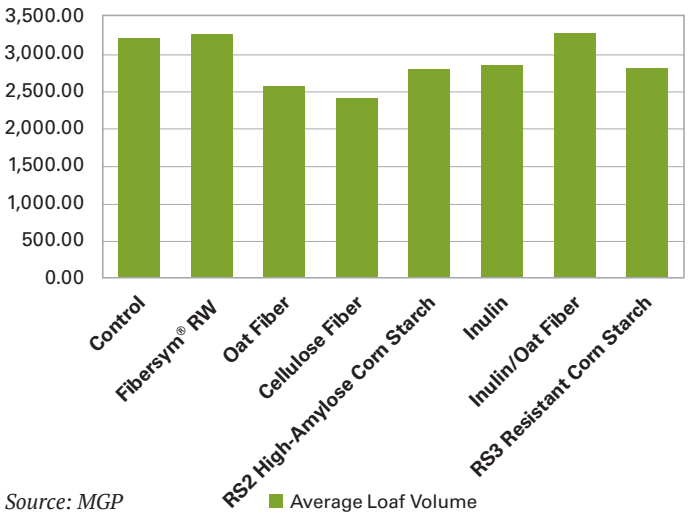
Proof times were about 17 to 20 minutes shorter, but bake times were 4 minutes longer compared to control doughs. HPHF breads displayed significantly greater volume (260 to 325 cc higher) than the corresponding control breads. Significant increases in moisture, protein, and dietary fiber along with reduction in calories were achieved using the HPHF formulas. Caloric reduction was adequate to meet the requirements for labeling as "reduced calorie" products. Texture analysis over a 10-day storage period demonstrated that the control bread firmed faster and more than the HPHF bread.

WHITE PAN BREAD

The performance in white bread of Fibersym® and other sources of dietary fiber was compared using a no-time dough formula. The bread flour in the control formula was substituted with an equal amount of fiber sample to achieve a dietary fiber level of 5 grams per 50 grams ("as is" moisture level) serving size of bread. Water absorption was varied to adjust for changes in water binding by the fiber samples. The doughs made with Fibersym, RS2 high-amylose corn starch, and RS3 resistant corn starch were the most convenient to process. Only slight changes in overall absorption level and mixing time were implemented. The doughs made with oat fiber and cellulose fiber required high water absorption and were challenging to work with to attain the targeted dietary fiber level. Inulin has lower water absorption (54%) than the control (63%), required longer mixing time, and water in the dough had to be added in four stages to produce an acceptable dough. Efforts to produce a suitable dough using a wheat dextrin as a source of fiber were unsuccessful.

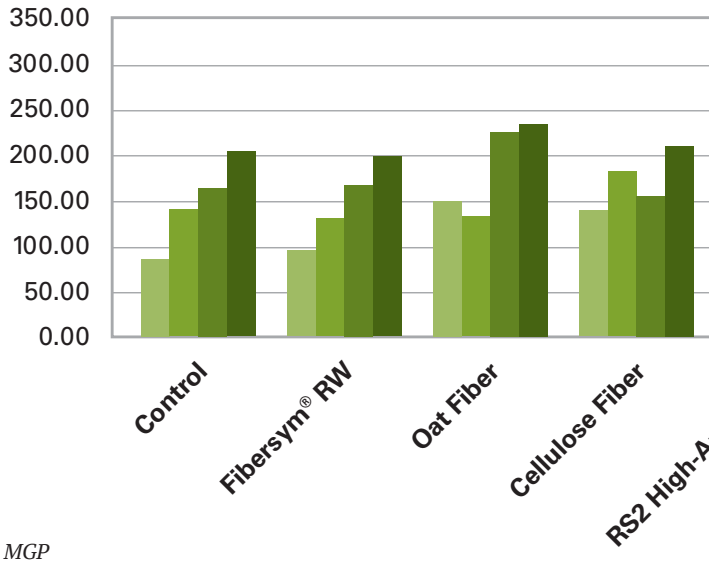
Dietary fiber has a weakening effect on the strength and structure of bread dough during proofing. This effect was most apparent with cellulose fiber. Its high water absorption and large particle size drastically weakened the strength of proofed dough. Inulin lengthened the proof time of the dough by 3.5 hours, which would require a significant increase in yeast level. The proofed strength and appearance of the dough formulated with Fibersym® looked identical to the control dough, whereas doughs containing RS2 high-amylose corn starch and RS3 resistant corn starch demonstrated slight weakness.

Figure 12. *Average loaf volume of breads formulated with different* ***fiber sources.***



Source: MGP

Figure 13. *Bread firmness after storage for 1, 4, 7 and 10 days.*



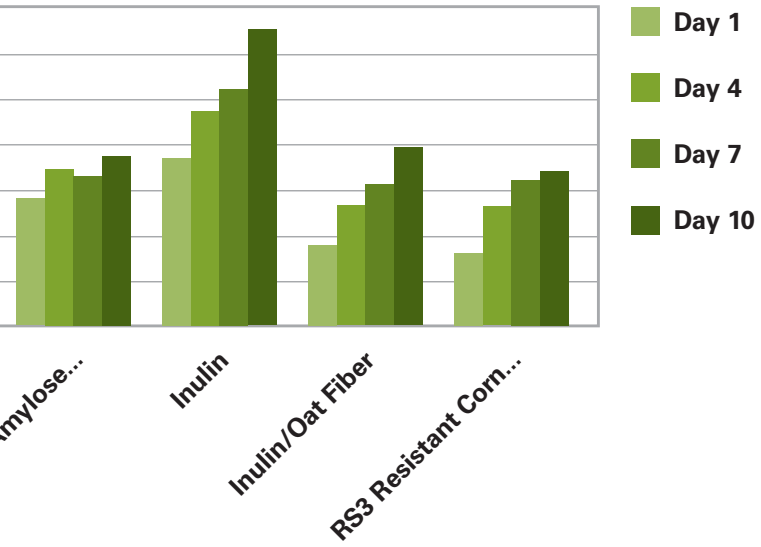
Source: MGP

Fibersym® required 22 minutes of baking at 410°F. The other fiber sources took the same length of baking time at 410°F, but inulin required baking for 28 minutes at 375°F to avoid excessive browning. Loaf volume for breads formulated with Fibersym® and a 1:1 blend of inulin and oat fiber exceeded the loaf volume of the control bread (Fig. 12).

The texture and eating quality of bread formulated with Fibersym® was similar to the control bread and was superior to the other bread samples. Both RS2 high-amylose corn starch and RS3 resistant corn starch yielded breads with very open texture and coarse crumb. Breads formulated with oat fiber and cellulose fiber also had a very open texture with large cell structures.

Furthermore, the breads had poor palatability and mouthfeel. Inulin imparted a tight crumb structure with a firm and dry texture. Changes in bread firmness were assessed by measuring the texture of bread samples after 1, 4, 7 and 10 days of storage. Fibersym® and other fiber sources, with the exception of inulin, are generally comparable in textural firmness compared to control bread (Fig. 13).

In a related study at Kansas State University, 1 part of Fibersym® was blended with 9 parts of wheat flour to produce white pan bread. A wheat starch formula used 1 part of wheat starch blended with 9 parts wheat flour, and a control formula used 100% wheat flour. The specific volume (5.96-6.07 cc/g) was slightly lower and the bread slightly firmer (427.8-475.2 g) for both the Fibersym® RW



and wheat starch formulas compared to the control wheat flour formula (6.28 cc/g; 406.9 g).

U.S. Patent Application 2009/0252843A1 describes a high fiber flour-based concentrate prepared by mixing stone ground whole wheat (44.4%), inulin (6.7%), wheat protein (8.9%) and Fibersym® (40%). A high fiber bread was made by mixing 1 part of high fiber, flour-based concentrate and 3 parts of bread flour. The resulting bread has a dietary fiber content of 8-12 g per 100 g serving and had natural lightness, good mouthfeel, and without the traditional heaviness of high fiber breads.

In a 2017 Kansas State University study, commercial bread flour was replaced with 0 (control), 5, 10, 15, 20 or 25% Fibersym. Vital wheat gluten was added to each flour-Fibersym® blend to maintain the original flour protein content of 11.8%. White pan bread was prepared from the blends using a straight dough procedure with a 90-min fermentation. The optimum mixograph water absorption of doughs containing 5, 10, and 15% Fibersym® was the same as the control with no added Fibersym; however, doughs with 20 and 25% Fibersym® has 2% higher absorption (**Table 12**). Doughs with added Fibersym® increased mixing time ranging from 15-45 sec. There was no significant difference in loaf volumes between the control bread and those with 5-20% Fibersym; however, the bread containing the highest level of Fibersym® (25%) had a significantly lower loaf volume compared with the control bread (**Table 12**). Bread firmness increased and elasticity decreased during the 14-day storage period; however, addition of Fibersym® had no influence on the firmness and elasticity values compared to the control. Overall liking score by average consumers was similar between control bread and Fibersym-containing bread. The researchers concluded that Fibersym® did not have a significant effect on dough development, strength, extensibility or handling properties or on bread volume, texture, shelf life or consumer acceptance.

In U.S. Patent 9,668,488, a technology to make a low calorie, high fiber food product with good taste properties is described. The total dietary fiber within the food product arising from RS4 wheat starch comprises 14-60% and the caloric count is in the range of 1 to 3.25 calories per gram on a dry weight basis. The food product may be bread, cookies, cakes, crackers, muffins, brownies, pizza crust, doughnuts, biscuits, pie, wafers, pasta, instant noodles, and egg noodles among others.

Table 12. *Effect of replacing flour by 5-25% of Fibersym® RW on dough and bread properties.*

Fibersym® RW, %	Water Absorption, %	Mix Time, min	Loaf Volume, cc	Overall Liking	Firmness after 14 days, g
0	62b	3.00d	845a	5.2a	1169a
5	62b	3.25c	829ab	-	1220a
10	62b	3.25c	804ab	-	1067a
15	62b	3.25c	799ab	4.8a	1139a
20	64a	3.50b	785ab	4.8a	1085a
25	*64a*	*3.75a*	*765b*	*5.1a*	*1111a*

Adapted from *Miller, R.A. and Bianchi, E. 2017. Effect of RS4 resistant starch on dietary fiber content of white pan bread. Cereal Chem. 94(2):185-189.*

FLOUR TORTILLAS

Flour tortillas represent one of the fastest growing categories of bakery products in the U.S. Because of their wide acceptance, they have moved from being an ethnic (Hispanic) food to being part of a typical American diet. To determine the effect of fiber fortification on properties and consumer acceptability of flour tortillas, a study was initiated by Texas A&M University (College Station, TX) using 5%, 10% and 15% levels of incorporation of Fibersym® RW. The processing of tortillas with 5%-15% Fibersym® generated doughs that were soft, extensible and easy to press. As shown in **Table 13**, the weights of Fibersym-fortified tortillas are practically similar to the control tortillas. Although the thickness tends to decrease with 10% and 15% usage levels, their diameters are significantly larger than the control tortillas. The thickness of tortillas with 5% Fibersym® is similar to the control tortillas, but the diameter tends to increase. These dimensional differences resulted in a calculated specific volume that tends to increase as the dosage of Fibersym® increases. The opacity of the flour tortillas tends to increase with the addition of Fibersym.

Freshly-made tortillas containing Fibersym® were softer and more tender than the control tortillas based on lower deformation modulus after texture analysis. Storage of tortillas for 16 days had the general effect of increased force to deform, decreased extensibility, and reduced work to crack, or rupture the tortilla. A sensory evaluation of one-day old tortillas showed comparable appearance and flavor, but texture was significantly more tender for 10% and 15% Fibersym-fortified tortillas compared to the control tortillas (**Table 13**). Tortillas with 15% Fibersym® had significantly higher overall acceptability scores than the control tortillas.

In a follow up study, scientists at Texas A&M University compared the properties of flour tortilla made from bread flour (15.5% protein) and tortilla flour (13.9% protein) that have been fortified with 15-25% Fibersym. Subjective and objective measurements revealed that increased substitution of tortilla flour with Fibersym® produced dough that was softer and less extensible whereas bread flour was not significantly affected. Due to its stronger gluten, the bread flour (control) produced smaller diameter tortillas than the tortilla flour (control). Substitution of flour with Fibersym® led to a significant increase in tortilla diameter for both flours, with a positive trend for level of Fibersym® substitution and diameter (**Table 14**). The added Fibersym® weakened the gluten matrix, created a less elastic dough with less shrink-back after hot-pressing leading to a larger diameter. Substitution of the flours with Fibersym® significantly increased its lightness (L* value) and specific volume of the tortillas (**Table 14**). Fibersym® weakened the gluten matrix of both tortillas, which led to better gas cell expansion during baking resulting in opacity.

Due to gluten dilution, the force to extend, rupture distance, and work to rupture tortillas were reduced with increased Fibersym® substitution. Fibersym-fortified bread flour tortilla exhibited remarkable shelf stability (flexibility during storage) over the 18-day storage period. In summary, fortification of bread flour with Fibersym® improved tortilla quality attributes. At Fibersym® substitution levels of 15-25%, the bread flour tortillas had textural properties similar to tortillas from tortilla flour, but had significantly better shelf stability.

Table 13. *Properties of flour tortillas fortified with Fibersym® RW.*

		Level of Fibersym® RW		
PARAMETER	CONTROL	5%	10%	15%
Weight, g	40.5	40.8	40.2	39.3
Thickness, mm	3.00	3.00	2.82	2.81
Diameter, mm	164	169	177*	176*
Specific Volume, cc/g	1.57	1.64	1.72	1.74
Opacity, %	75	85	88	90
Texture Score	6.7	7.1	7.8*	8.2*
Overall Acceptability Score	6.6	6.3	7.0	7.5*

*Indicates significant difference from control (P<0.05)

Adapted from *Alviola, J.N., Jondiko, T., and Awika, J.M. 2010. Effect of cross-linked resistant starch on wheat tortilla quality. Cereal Chem. 87:221-225.* Used with permission.

Table 14. *Effect of incorporating 15-25% of Fibersym® RW to tortilla flour and bread flour on properties of flour tortilla.*

Fibersym® RW (%)	Thickness (mm)	Diameter (mm)	Specific Volume (cm^3/g)	Opacity (L*)
TORTILLA FLOUR				
0	3.04b	158a	1.4a	80.3b
15	2.84ab	171b	1.6b	81.9c
20	2.79a	178bc	1.7b	83.2cd
25	2.69a	182c	1.7b	83.7d
Overall Mean	2.84	172	1.6	82.3
BREAD FLOUR				
0	3.05b	155a	1.4a	76.6a
15	2.99b	164ab	1.5ab	79.5b
20	2.91b	170b	1.6b	81.2bc
25	2.95b	174b	1.7b	82.0c
Overall Mean	2.97	166	1.5	79.8

Adapted from *Alviola, J.N, Jondiko, T.O., and Awika, J.M. 2012. Effect of strong gluten flour on quality of wheat tortillas fortified with cross-linked resistant starch. J. Food Proc. Preserv. 36:38-45.*

SUGAR-SNAP COOKIES

A study conducted by Kansas State University compared the performance of Fibersym® and RS2 potato starch in sugar-snap cookies. Both starches produced cookies with top grain and spread factors comparable to the control flour (**Table 15**). However, cookies made with Fibersym® exhibited similar snapping force and RS2 potato starch demonstrated significantly lower snapping force when compared to the control cookie.

Table 15. *Properties of sugar-snap cookie formulated with Fibersym® RW and potato starch.*

	Spread Factor	Top Grain	Snapping Force, kg
Flour (Control)	107.9a	Good	9.22a
Fibersym® RW/Flour (1:9)	110.8a	Good	9.30a
Potato Starch/Flour (1:9)	113.8a	Good	7.35b

Note: Values followed by different letters in the same column are significantly different at 5% level.

Adapted from *Yeo, L.L. and Seib, P.A. 2009. White pan bread and sugar-snap cookies containing wheat starch phosphate, a cross-linked resistant starch. Cereal Chem. 86:210-220. Used with permission.*

EXTRUDED BREAKFAST CEREALS

To study the effect of extrusion on fiber retention of Fibersym® when formulated in breakfast cereals, a study was conducted at Wenger Technical Center (Sabetha, KS). Five blends consisting of a control blend and four other blends differing in the levels of Fibersym® were prepared. The control blend consisted of 42% whole corn flour, 30% long grain rice flour, 20% whole oat flour, 6% sugar and 2% salt. The four treatment blends were formulated with Fibersym, replacing 5%, 10%, 15%, and 20% of whole corn flour with the other ingredients remaining the same. Ring-shaped breakfast cereals were prepared in duplicate from the blends using a TX-57 twin-screw extruder at approximately similar processing conditions with an extruder shaft speed of 200 rpm and die temperature of 118°C. Both the blends and the extruded breakfast cereals were analyzed by an outside laboratory for moisture and total dietary fiber by AOAC Method 991.43. Fiber content on a dry basis and fiber retention are depicted in **Table 16**.

The total dietary fiber content of the dry ingredient blends increased by roughly 3.6% for every 5% added Fibersym. Total dietary fiber loss during extrusion processing increased as Fibersym® level increased; however a high percentage (78 – 89%) of the total dietary fiber content was retained in the final product. Product density (**Table 17**) increased as level of Fibersym® increased but no effect on the specific mechanical energy was observed. X-ray microtomography showed that Fibersym® did not affect internal cell wall thickness, size or porosity. Addition levels of 5 and 10% had no effect on expansion, physical appearance, initial crispness or bowl life of the cereal rings. Higher levels of incorporation (15% and 20%) decreased cereal ring diameter, but increased initial (dry) product crispness and extended bowl life (**Table 17**). In general, moisture content and moisture uptake of the cereal rings during soaking in milk was not affected by the level of Fibersym® in the formula. Furthermore, moisture content and moisture uptake did not appear to influence the crispness of milk-soaked cereal rings.

In U.S. Patent 8,563,065, extruded, directly expanded, high-fiber reduced calorie ready-to-eat cereal was produced by replacing 50% of the total flour weight in the formula with Fibersym. Compared to the control cereal with 9.73% moisture and 0.452 g/cm3 bulk density, the Fibersym-containing cereal has 7.81% moisture and a lighter bulk density of 0.365 g/cm3. The Fibersym® cereal is lighter in color, less red and slightly more yellow than the control cereal. Air cells were more numerous and larger in the control. Brittleness was comparable in both cereal products.

Table 16. *Survivability of Fibersym® RW during extrusion of a ring-shaped breakfast cereal.*

Level of Fibersym® RW	% Fiber (d.b.) of Dry Blend (Before Extrusion)	% Fiber (d.b.) of Extruded Breakfast Cereal	% Fiber Retention
0%	6.4	5.6	88.1
5%	10.6	9.4	88.7
10%	14.2	11.6	81.7
15%	18.0	14.8	82.2
20%	21.3	16.6	77.9

Adapted from *Miller, R.A., Jeong, J., and Maningat, C.C. 2011. Effect of RS4 resistant starch on extruded Ready-to-Eat (RTE) breakfast cereal quality. Cereal Chem. 88(6):584-588.*

Table 17. *Properties of ring-shaped, extruded breakfast cereals formulated with different levels of Fibersym® RW*

Level of Fibersym® RW, %	Product density, kg/m3	Diameter, mm	Crispiness of dry cereal ring, g	Crispiness of cereal rings soaked in milk (5 min.), g
0	66	15.17	8282	314
5	72	14.67	7835	438
10	73	14.05	8963	438
15	84	13.23	9398	784
20	86	13.15	11040	992

Adapted from *Miller, R.A., Jeong, J., and Maningat, C.C. 2011. Effect of RS4 resistant starch on extruded Ready-to-Eat (RTE) breakfast cereal quality. Cereal Chem. 88(6):584-588.*

PASTA

In conjunction with the addition of Arise® 6000, a wheat protein isolate produced by MGP, the functionality of Fibersym® RW in spaghetti was evaluated in a study conducted at North Dakota State University (Fargo, ND). Dry (raw) spaghetti made from a blend of 1.7-3.4% Arise® 6000, 12.6-12.8% Fibersym, and 84.0-85.5% semolina increased in brightness (L* value) but yellowness (b* value) was unaffected compared to the control spaghetti (100% semolina). Water absorption of cooked spaghetti decreased from 3.1 g/g for the control to 2.8-2.9 g/g for the spaghetti formulated with Arise® 6000 and Fibersym. Cooking loss tended to decrease from 4.4% (control spaghetti) to 3.7% for spaghetti containing 3.4% Arise® 6000 and 12.7% Fibersym, while the eating texture tended to get firm with the addition of 2.5-3.4% Arise® 6000 and 12.6-12.7% Fibersym®(6.4-6.5 gcm) compared to the control spaghetti (6.0 gcm).

In a second study at North Dakota State University, a higher level of Fibersym® (48%) and Arise® 6000 (12%) was blended with semolina (40%) and processed into spaghetti. As shown in **Table 18**, the raw spaghetti formulated with Fibersym® and Arise® 6000 decreased in yellowness, but increased in brightness compared to the control spaghetti. Cooking time increased by three minutes, but both water absorption and cooking loss decreased for the spaghetti formulated with Fibersym® and Arise® 6000. Texture analysis revealed an increase in firmness upon incorporation of the two additives.

Table 18. *Properties of raw and cooked spaghetti formulated with Fibersym® RW and Arise® 6000.*

PARAMETERS	SPAGHETTI SAMPLE	
	100% Semolina (Control)	40% Semolina, 12% Arise® 6000, 48% Fibersym® RW
RAW SPAGHETTI		
Brightness (L value)	60.6	66.2
Yellowness (b value)	4.0	3.4
COOKED SPAGHETTI		
Cooking Time, min	11.0	14.0
Water Absorption, g/g	2.8	2.3
Cooking Loss, %	5.6	4.0
Firmness, gcm	7.2	8.0

Source: MGP

In yet another study, the addition of egg white powder was evaluated in conjunction with the use of Fibersym® and Arise® 6000 in a spaghetti formula. The color of dry spaghetti was slightly affected as the brightness tended to decrease (L* value: 60.1 versus 59.0) and yellowness tended to increase (b* value: 39.8 versus 40.7) compared to the control spaghetti. As expected, cooking time increased and both water absorption and cooking loss decreased (**Table 19**). A significant increase in firmness of cooked spaghetti was observed.

Table 19. *Cooking properties of spaghetti containing Fibersym® RW, Arise® 6000, and egg white powder*

PARAMETERS	SPAGHETTI SAMPLE	
	100% Semolina (Control)	81% Semolina, 12% Fibersym® RW, 2% Arise® 6000, 5% Egg White Powder
Cooking Time, min	9.25	10.0
Water Absorption, g/g	3.0	2.6
Cooking Loss, %	5.2	4.4
Firmness, gcm	5.8	12.2

Source: MGP

In U.S. Patent Application 2009/0252844A1, a high fiber pasta was made using a blend of high fiber wheat flour component and durum flour. The high fiber wheat flour component consists of 44.4% stone ground whole wheat, 6.7% inulin, 8.9% wheat protein and 40% Fibersym. Pasta was made from 1 part high fiber wheat flour component and 2.5 parts durum flour using 27% water absorption. The finished high fiber pasta has 12 g dietary fiber and 7 g protein per 57 g serving size. The cooked pasta has "al dente" mouthfeel and a pleasant taste without grainy texture.

ASIAN NOODLES

White salted noodles, Chinese-style noodles (Chuka-men) and instant fried noodles formulated with a blend of Fibersym® and Arise® 6000 (84:16 ratio) were prepared in the laboratory of Wheat Marketing Center (Portland, OR). Noodle flour was substituted by a 10% and 30% of Fibersym/Arise® 6000 blend.

During Asian noodle making, all of the above noodle doughs have acceptable processability and machinability. Incorporation of the Fibersym/Arise® 6000 blend in white salted noodles tends to increase lightness, but tends to decrease yellowness. The yellowness of Chuka-men noodles tends to decrease as the level of substitution increases. The color of Chuka-men noodles was acceptable at a 10% substitution level only. The instant fried noodles have acceptable lightness and yellowness. The addition of Fibersym/Arise® 6000 tends to decrease the water absorption of cooked noodles for all three noodle types. Using a TA.XT2 texture analyzer, all Asian noodles formulated with Fibersym/Arise® 6000 at 10% and 30% levels have acceptable bite, springiness, and mouthfeel.

SNACKS

Indirect expanded snacks formulated with 5% and 10% Fibersym® were prepared in the Food Processing Center of the University of Nebraska (Lincoln, NE). The control snack contained 30% tapioca starch, 58% wheat flour, 10% corn flour, 0.5% monoglycerides, 1% salt and 0.5% sodium bicarbonate. Fibersym® was incorporated by replacing 5% or 10% of tapioca starch while the rest of the ingredients were kept the same. Snack pellets were prepared using a TX-57 twin-screw extruder, and the dried pellets were expanded by frying in oil. The expanded snack products containing Fibersym® appeared lighter in color compared to the control. Due to less axial and radial expansion, the Fibersym-formulated snacks had heavier bulk density than the control. Interestingly, fat absorption was decreased from 26% for the control to 19%-22% upon the addition of 5% or 10% Fibersym. In addition, Fibersym® increased the crispiness of the snack products as indicated by the higher linear distance and higher mean number of major peaks when measured by the TA.XT2 Texture Analyzer (**Table 20**).

Expanded products by extrusion technology with high fiber and protein contents are described in U.S. Patent 8,741,370. Fibersym® was added at a 5-10% level into a dry mix containing wheat protein isolate (60-70%), oxidized wheat starch (20.6-28.6%), sodium bicarbonate (1%), sodium aluminum phosphate (0.5%), sodium aluminum sulfate (0.5%), calcium aluminum phosphate (0.5%), Vitamin E (0.14%), water (15%) and vinegar (2%). The mixture was extruded to yield an expanded product with ~2% moisture, bulk density of 14-17 lb/ft3, protein of 52-61% and total dietary fiber of 4.3-7.5%.

Table 20. *Crispiness texture analysis of indirect expanded snacks formulated with Fibersym® RW.*

Crispiness Parameters		
LEVEL OF FIBERSYM® RW	LINEAR DISTANCE	MEAN NUMBER OF MAJOR PEAKS
0%	1949	17.0
5%	2417	26.8
10%	2251	23.5

Source: MGP

TEXTURED CRUMBS

Texturizers like bread crumbs are normally used in coating frozen or refrigerated ready-to-cook food products to provide desirable appearance and crispiness attributes. However, the desired appearance and textural qualities of a freshly fried food is difficult to achieve/maintain because of extreme processing conditions (e.g. refrigerated or frozen storage, freeze-thaw cycles, heating in warming cabinets or heat lamps, and re-heating in conventional or microwave ovens). To overcome these challenges, Australian Patent AU 2014280802B2 describes the preparation of textured crumb products containing Fibersym® by high pressure short time extrusion. In a wheat flour-based formulation, textured crumbs with 10-20% Fibersym® showed intact starch granules of Fibersym® which exhibited a glassy, friable state even at 44% moisture. The glass transition temperature,Tg, of the Fibersym-containing crumbs occurred between about 50-60°C. The textured crumbs with Fibersym® exhibited crispy and fracturable features in a chicken nugget application where the product was subjected to freeze/thaw cycling and microwave reheating.

The influence of substitution of wheat flour by Fibersym® in a batter formula for coating black pomfret fillets was studied by scientists at Universiti Putra Malaysia. A significant increase ($p<0.05$) of water retention capacity was demonstrated in batters substituted at 10 and 20% Fibersym, which was reflected in the moisture content of the breaded fillets. The addition of Fibersym® significantly decreased ($p<0.05$) the fat content of the breaded fillets by 2-3% after frying, and also resulted in significant increases in hardness and fracturability of the products. L* and b* values increased significantly ($p<0.05$) in the samples containing 20% Fibersym® as compared to the control. Micrographs of the cross-section of the breaded crusts with Fibersym® showed a more compact structure reflecting the observed trend in the hardness values.

CONFECTIONERIES

To create healthy, indulgent confectionery products, Fibersym® RW was successfully formulated at a level of ~5% in crème, caramel, marshmallow, and sugar-free confectionery bars. Fibersym® RW is comprised of microscopic, smooth-surfaced particles that contributed to the smooth mouthfeel of the finished product, and it was easily dispersed in the formulas with no clumping issues. The sensory attributes of flavor, texture, and color of the four confectionery products were comparable to the control formulas.

PET FOOD PRODUCTS

Fibersym® at 31.8% level was formulated in a low calorie starch-based pet chew product together with other ingredients consisting of pre-cooked corn flour, palatant, glycerol monostearate, vegetable oil, magnesium stearate, anti-oxidant mix, glycerine and water as reported in U.S. Patent Application 2006/0193959A1. The mixture was extruded into pellets, which were subsequently fed into an injection molding press. The pet chew product exhibited a whitish surface appearance and acceptable texture.

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