

## Increasing Dietary Fiber in Foods: The Case for Phosphorylated Cross-Linked Resistant Starch, a Highly Concentrated Form of Dietary Fiber

- ▶ Commercially available cross-linked resistant wheat starch typically exhibits  $\geq 90\%$  dietary fiber (average 95% and minimum 85%, dry basis) and  $>80\%$  resistant starch when assayed, respectively, by the Total Dietary Fiber Method 991.43 of AOAC International and the Englyst method.
- ▶ Healthy young adults ( $n = 13$ ) consuming nutrition bars made with cross-linked resistant wheat starch, as opposed to a puffed wheat (control), showed decreased blood glucose and insulin levels, even though both bars were matched at 50 g of available carbohydrate. The test bar contained 20 g of total dietary fiber and 14 g of resistant starch, whereas the control bar contained 5 g of total dietary fiber.
- ▶ Consumption of a cracker formulated with cross-linked resistant wheat starch over a three-week period at a calculated intake of 26 g/d of dietary fiber from the resistant starch was well tolerated and rapidly (2–4 d) increased bifidobacteria by more than three fold in 10 healthy human subjects (male and female).
- ▶ Cross-linked resistant starch appears to deliver many of the health benefits of dietary fiber without negatively impacting organoleptic and textural properties across a wide range of food products.

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In the last 25 years, obesity has increased dramatically in the United States. According to a 2007 National Health Interview Survey on Body Mass Index (51), more than one-third of U.S. adults aged 20 years and older, or 72 million people, are obese with an equal percentage among men and women. Obesity is of global concern as well; currently, more than 15% of adults and 10% of children in the world are estimated to be overweight or obese (22). The increasing rates of excessive weight and obesity raise serious concerns for human health. Obesity has been related to the risk of many diseases, including coronary heart disease, hypertension, respiratory complications, osteoarthritis, and especially type 2 diabetes. Type 2 diabetes leads to serious health problems, including heart disease, blindness, nerve damage, and kidney failure. According to the American

Diabetes Association (5), currently 23.6 million children and adults in the United States, or 7.8% of the population, have diabetes, and of those about 90% have type 2 diabetes. The twin epidemics of obesity and type 2 diabetes are caused by excessive energy intake from calorie-dense foods and by decreased energy expenditure from a sedentary lifestyle (12,23). There is a rising and urgent need to develop foods with reduced energy density, and for adults and children to increase their physical activity.

### Dietary Fiber and Resistant Starch

Dietary fiber has been recognized as a desirable component of food to reduce the risks of obesity and type 2 diabetes, as well as to provide other health benefits, such as colonic health. The intake of fiber-rich food helps to reduce caloric intake and appears to prolong satiety by delayed emptying of the stomach. Clinical studies (31,38,42) on fiber-rich diets show improved glycemic control, increased insulin sensitivity, reduced serum lipids, and decreased blood pressure. In the early 1980s, researchers discovered that some starch escapes digestion in the upper gastrointestinal tract of humans and they termed it resistant starch (9,18). Resistant starch is currently defined as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (7). Resistant starch is recognized as dietary fiber and occurs in food as

four classes: type 1—physically inaccessible starch; type 2—granular starch with B-type crystals; type 3—retrograded amylose; and type 4—chemically modified starches. Resistant starches of all types are believed to function in many ways as dietary fiber in the human digestive tract (48,50).

### Definition and Labeling of Dietary Fiber

The U.S. Food and Drug Administration has no official definition for dietary fiber. However, the Nutritional Labeling and Education Act (NLEA) of 1991 specified that the dietary fiber content of a food is to be measured for the purpose of its nutritional labeling according to the Association of Official Analytical Chemists (AOAC) International’s Total Dietary Fiber in Foods, Enzymatic Gravimetric Method, or its modifications (8,20). Resistant starch is analyzed as dietary fiber by the approved AOAC International official method, and satisfies the requirement for NLEA when a claim of dietary fiber is made on a food. In 2001, AACC International proposed the definition of dietary fiber as being “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glu-

cose attenuation” (1). In this definition of dietary fiber, resistant starch is included under analogous carbohydrates.

In 2001, the Institute of Medicine (IOM) of the National Academy of Sciences in the United States proposed the following definition: “Dietary Fiber consists of non-digestible carbohydrate and lignin that are intrinsic and intact in plants. Functional fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans. Total dietary fiber is the sum of dietary fiber and functional fiber” (26,27). Under the IOM definition, resistant starch is classified as functional fiber.

In 2006, the definition and properties of dietary fiber were agreed upon at the 28th session of the CODEX Committee on Nutrition and Food for Special Dietary Uses, CODEX Alimentarius Commission, FAO/WHO (16,21). The CODEX definition reads “dietary fiber means carbohydrate polymers with a degree of polymerization (DP) not lower than 3, which are neither digested nor absorbed in the small intestine. A degree of polymerization not lower than 3 is intended to exclude mono- and disaccharides. It is not intended to reflect the average DP of a mixture. Dietary fiber consists of one or more of: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers that have been obtained from food raw material by physical, enzymatic, or chemical means; and synthetic carbohydrate polymers.” As described by CODEX, “Dietary fiber generally has properties such as: decrease of intestinal transit time and increase of stool bulk; fermentable by colonic bacteria; reduce blood total and/or LDL cholesterol levels, and reduce postprandial blood glucose and/or insulin levels.” According to the CODEX definition, resistant starch is included in the definition of dietary fiber since it is obtained from raw food material by physical, enzymatic, or chemical means and has the general properties specified in the definition.

### Resistant Starch with a High Level (≥ 90%) of Total Dietary Fiber

In 1999, phosphorylated cross-linked starch was introduced (46,54) as a type 4 resistant starch (RS4). Phosphorylated cross-linked RS4 is a highly concentrated form of dietary fiber that may be produced from any starch, including wheat, normal corn, high-amylose corn, potato, tapioca, oat, and banana. The cross-linked resistant starch fits within the RS4 category and exhibits up to 100% dietary fiber when assayed by the AOAC International Total Dietary Fiber Method 991.43 (6,13,37,43, 44,60). In a six-week animal feeding study,

hamsters fed a diet containing 10% phosphorylated cross-linked resistant wheat starch showed: a) lower feed consumption and weight gain; b) a higher level of high-density lipoprotein cholesterol in the blood; c) a lower level of liver cholesterol; and d) a higher level of colonic short-chain fatty acids, especially butyric acid, compared to hamsters fed a 10% cellulose diet (46,54). In a human study with healthy young adults eating a nutritional bar formulated with phosphorylated cross-linked RS4 compared to a bar formulated with an equal amount of puffed wheat, blood glucose response, insulin response, C-peptide concentration, and pancreatic  $\beta$ -cell insulin secretion rate were reduced and insulin

sensitivity by the Matsuda insulin sensitivity index was increased (4).

### Government Regulations

Phosphorylated cross-linked RS4 is produced commercially from wheat, potato, and tapioca starches in compliance with the Code of Federal Regulations (CFR) in the United States (14). According to those regulations, the cross-linked resistant starch falls in the category of “food starch modified” and is chemically defined as “phosphated distarch phosphate.” There is no use-level limitation for food-grade cross-linked starch according to 21 CFR 172.892; and it may be safely used under good manufacturing practices.

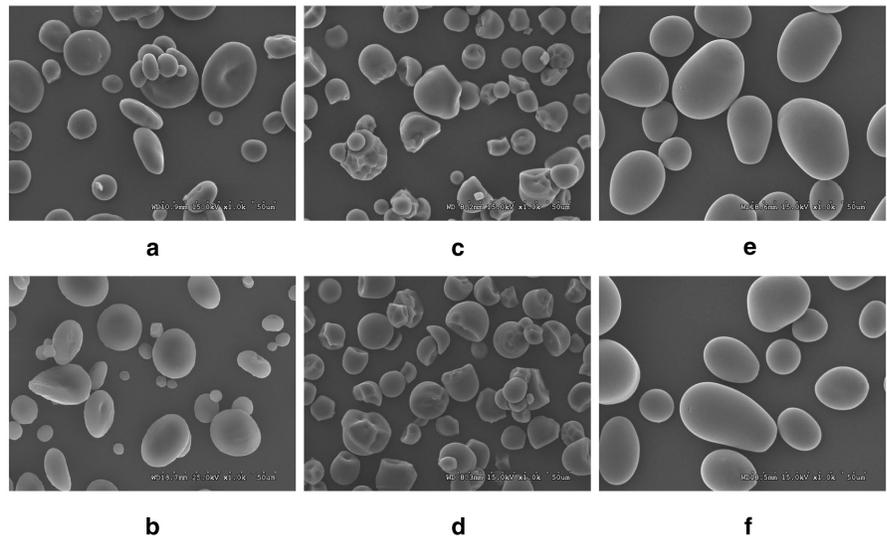


Fig. 1. Scanning electron microscopic (SEM) images ( $\times 1,000$ ) of a) native wheat starch, b) cross-linked resistant wheat starch, c) native tapioca starch, d) cross-linked resistant tapioca starch, e) native potato starch, and f) cross-linked resistant potato starch.

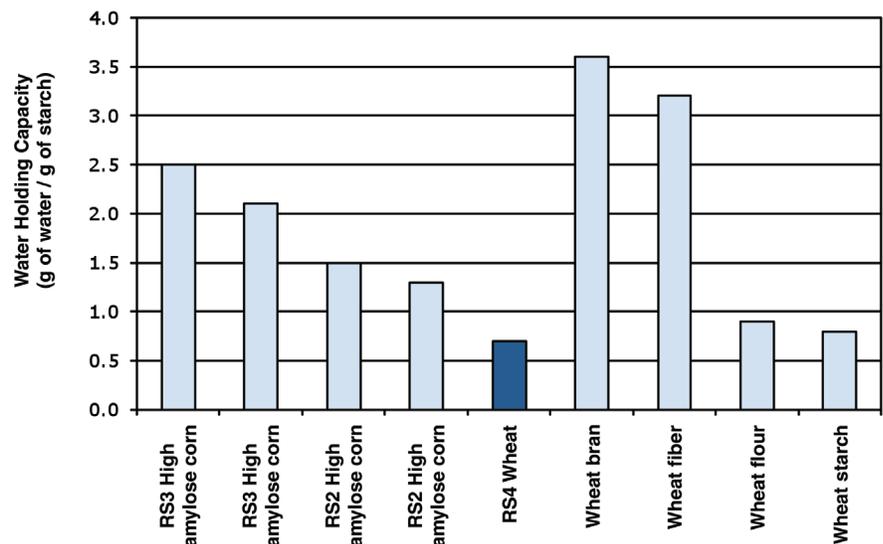


Fig. 2. Water-holding capacity of cross-linked resistant wheat starch compared with those of conventional fibers, commercial type-2 and type-3 resistant starches, wheat flour, and wheat starch.

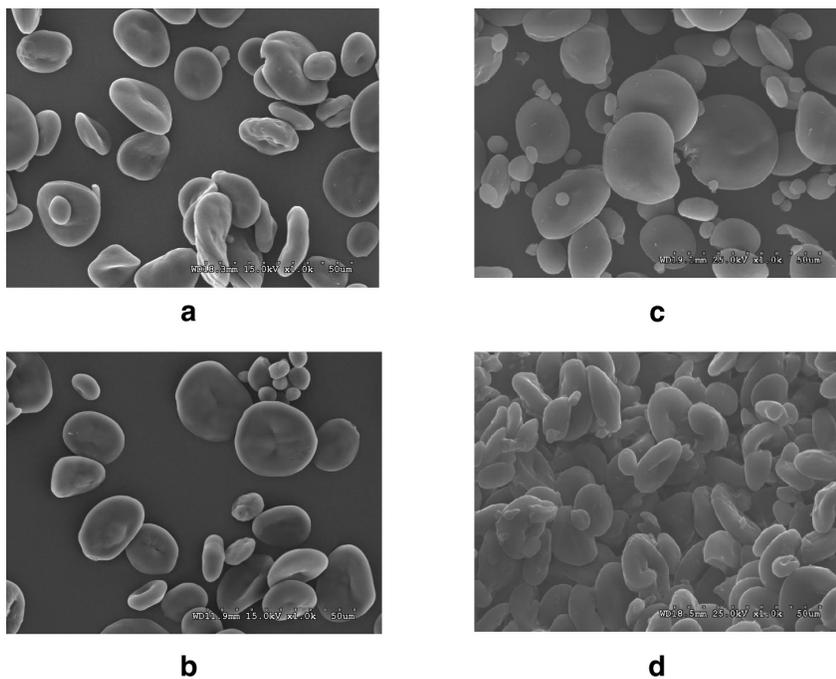
The Scientific Committee for Food of the European Economic Community (45) concluded in "Reports of the Scientific Committee for Food (Thirteenth Series)," that the committee is of the opinion that modified starches in Group B, which include phosphorylated distarch phosphate, can be regarded as acceptable and the committee considered it unnecessary to establish the individual acceptable daily intakes (ADIs) provided technological usage remained at present-day levels. According to the IOM, there are no reports of adverse effects resulting from high dietary phosphorus (phosphate) intake in humans (28). The IOM calculated a safe upper level of 4.0 g of phosphorus a day for adults aged

9 to 70 years old and 3.0 g of phosphorus a day for children 1 to 8 years old and persons 70 or more years old. Phosphorylated cross-linked RS4 contains a low level of phosphorus, less than 0.5 g per 100 g of starch. Consuming 50 g of phosphorylated cross-linked resistant starch daily would provide approximately the same amount of phosphodiester (equivalent to ~0.2 g phosphorus) that humans consume as DNA or RNA in their diet, which is ~1 g of each daily with both containing ~10% phosphorus (30). The Joint Expert Committee on Food Additives of the FAO/WHO (61,62) considered phosphated distarch phosphate suitable for use in foods without specifying an ADI. This is in ac-

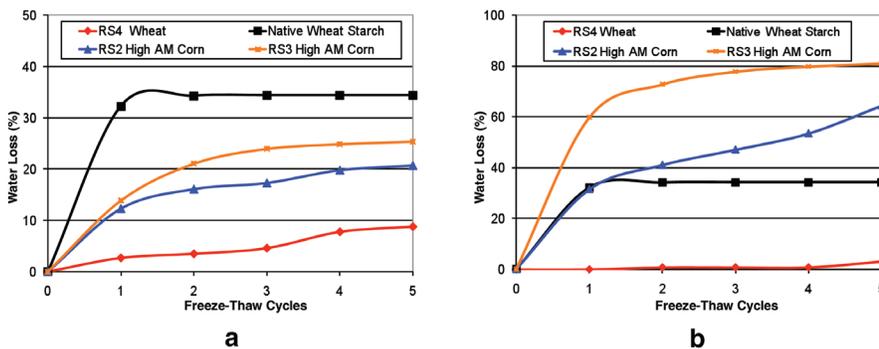
cordance with the position taken by regulatory groups in the United States, Canada, and the United Kingdom.

### Glycemic Properties

Commercially available cross-linked resistant wheat starch typically exhibits  $\geq 90\%$  dietary fiber (average 95% and minimum 85%, dry basis) when assayed using the AOAC International Total Dietary Fiber Method 991.43 (40,55). For food labeling according to "Nutritional Labeling of Foods," published in Title 21 CFR 101.9 (15), cross-linked resistant wheat starch typically is calculated to contain less than 0.4 kcal/g. The cross-linked resistant wheat starch was shown to contain about 10% slowly digestible starch (SDS) and 80% of resistant starch (RS) by the in vitro assay of Englyst and coworkers (17,41). A high proportion of SDS and RS relative to rapidly digestible starch or rapidly available glucose is an indication of a low glycemic ingredient or food. After Jenkins et al. (32) developed the concept of glycemic index (GI), GI has been a useful tool for determining the speed at which the carbohydrates in food are digested and absorbed. GI is defined as the area under the blood glucose response curve after the consumption of 50 g of available carbohydrate from a test food divided by the area under the blood glucose curve after the consumption of the same amount of glucose standard (53). Available carbohydrate in a food is that fraction of carbohydrate that is not dietary fiber. In general, foods with a GI value above 70 are considered high GI foods, whereas foods with a GI value of 55 to 70 or a GI value lower than 55 are classified as medium GI foods and low GI foods, respectively (12,52). A low glycemic food is considered to be beneficial for health, especially for individuals afflicted with obesity and type 2 diabetes. Short- and long-term studies in humans and animals indicate that high glycemic diets affect appetite and nutritional partitioning for promotion of fat storage. After a high glycemic food intake, ratings of hunger during the postprandial period were higher than those after low to medium glycemic diets, which was evidenced by increased voluntary food intake after a high glycemic diet (2,10,11,25,34,35). The GI of a bakery food containing resistant starch has been determined by two methods. In one method, subjects consumed test foods that contained 50 g of available carbohydrates plus ~9 g of RS2 or RS3 in comparison to the reference white bread with 50 g of available carbohydrates (33). In the other method, subjects consumed test and reference foods containing the same amount of total car-



**Fig. 3.** Scanning electron microscopic (SEM) images ( $\times 1,000$ ) of a) cross-linked resistant wheat starch in excess water after conventional cooking at 95°C for 30 min; b) autoclaving at 120°C for 30 min; c) two-stage homogenization at 2,000 psi (13,800 kPa) and 500 psi (3,500 kPa), followed by pasteurization at 81°C for 20 s; and d) extrusion at specific mechanical energy at 203 kJ/kg.



**Fig. 4.** Freeze-thaw stability (syneresis) of cross-linked resistant wheat starch compared with native wheat starch and commercial type-2 and type-3 resistant high-amylose corn starches. Prior to freeze and thaw cycles, the starches in water (5%, w/w) were subjected to a) conventional cooking at 95°C for 30 min or b) autoclaving at 120°C for 30 min.

bohydrates that included dietary fiber (3,24).

In a recent clinical study (4) with 13 healthy young adults, breakfast bars were formulated with the same and equal levels of ingredients, but the test bar contained phosphorylated cross-linked wheat starch (RS4) instead of puffed wheat (reference bar). In a cross-over experimental design, the subjects consumed 50 g of available carbohydrate from the two bars and a glucose solution. Upon comparing integrated blood glucose levels, the glycemic index was calculated to be 19.9 for the test bar and 59.9 for the reference bar. Those results suggest that the cross-linked RS4 offers a convenient way to lower the GI of a carbohydrate-rich food, thereby leading to improved management of body weight and type 2 diabetes.

### Health-Promoting Food Products

The IOM recommended a daily intake of 25 g of dietary fiber for adult women and 38 g for adult men (29). However, most Americans consume less than 15 g of fiber each day. To increase the consumption of fiber, foods are being supplemented with conventional sources of fiber. Typical sources of fiber in the human diet are unrefined grains, fruits, and vegetables. Conventional fiber supplements produced from most sources need to be refined and ground to improve color and flavor and to eliminate a rough mouth-feel. Moreover, the fortification of bakery products with conventional dietary fiber often requires substantial formulation and process changes because of its rate of hydration and high water absorption. In most bakery applications, a pure and concentrated form of fiber with minimal water absorption is preferred (19). The low water absorption is also important in managing retrogradation and water activity of a finished food product.

Phosphorylated cross-linked RS4 is an attractive source of dietary fiber because it is white in color, neutral in flavor, smooth in texture, absorbs little water, and causes minimal rheological changes in a food product during and after processing. Commercially available cross-linked resistant starches are essentially identical in granule size, shape, and color to their parent starches (Fig. 1). They may be considered “invisible” sources of dietary fiber, especially when wheat-based resistant starches are formulated in flour-based foods (36,54,59,60). Cross-linked resistant wheat starch absorbs less water (<0.7 g/g of starch) than most conventional sources of dietary fiber (Fig. 2) and is considered a preferred source of fiber for bakery products. Cross-linked resistant wheat starch reduces reformulation challenges related

to excessive water uptake when making nutritionally enhanced bakery products and helps to achieve labeling claims of “good source of fiber” (>2.5 g of fiber per serving) or “excellent source of fiber” (>5.0 g of fiber per serving). White pan bread with 30% replacement of flour with phosphorylated cross-linked RS4 (63) and heat-moisture treated high-amylose (70%) corn starch (65) gave specific loaf volumes of 5.9 cc/g and 4.4 cc/g, respectively, compared to the control bread of 6.3 cc/g. The test bread with RS4 contained 21% (dry solid basis) dietary fiber compared to 3.3% for the control bread, and 50 g (“as is”) of test bread would provide 6.3 g of dietary fiber with ~11% fewer calories (64).

Cross-linked resistant wheat starch

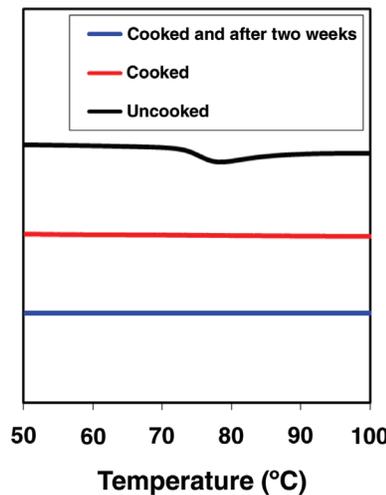


Fig. 5. Differential scanning calorimetry measurements on cross-linked resistant wheat starch in three parts of water before cooking and after storing for two weeks at 4°C.

	To	Tp	Tc	ΔH
	(°C)			(J/g)
Uncooked	74.9	80.8	94.1	14.5
Cooked (95°C for 30 min)	--	--	--	--
Cooked and after two weeks at 4°C	--	--	--	--

Control Bread	
<b>Nutrition Facts</b>	
Serving Size 2 slices (50g) Servings Per Container 10	
Amount Per Serving	
<b>Calories 120</b>	<b>Calories from Fat 15</b>
% Daily Value*	
<b>Total Fat 1.5g</b>	<b>3%</b>
<b>Saturated Fat 0g</b>	<b>0%</b>
<b>Cholesterol 0mg</b>	<b>0%</b>
<b>Sodium 230mg</b>	<b>10%</b>
<b>Total Carbohydrate 22g</b>	<b>7%</b>
<b>Dietary Fiber less than 1g</b>	<b>3%</b>
<b>Sugars 1g</b>	
<b>Protein 4g</b>	
<b>Vitamin A 0%</b>	<b>Vitamin C 4%</b>
<b>Calcium 2%</b>	<b>Iron 8%</b>
*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:	
Calories: 2,000 2,500	
Total Fat	Less than 65g 80g
Saturated Fat	Less than 20g 25g
Cholesterol	Less than 300mg 300mg
Sodium	Less than 2,400mg 2,400mg
Total Carbohydrate	300g 375g
Dietary Fiber	25g 30g
Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4	

Fiber-Fortified Bread	
<b>Nutrition Facts</b>	
Serving Size 2 slices (50g) Servings Per Container 10	
Amount Per Serving	
<b>Calories 100</b>	<b>Calories from Fat 15</b>
% Daily Value*	
<b>Total Fat 1.5g</b>	<b>3%</b>
<b>Saturated Fat 0g</b>	<b>0%</b>
<b>Cholesterol 0mg</b>	<b>0%</b>
<b>Sodium 230mg</b>	<b>10%</b>
<b>Total Carbohydrate 28g</b>	<b>8%</b>
<b>Dietary Fiber 5g</b>	<b>19%</b>
<b>Sugars 1g</b>	
<b>Protein 4g</b>	
<b>Vitamin A 0%</b>	<b>Vitamin C 4%</b>
<b>Calcium 2%</b>	<b>Iron 6%</b>
*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:	
Calories: 2,000 2,500	
Total Fat	Less than 65g 80g
Saturated Fat	Less than 20g 25g
Cholesterol	Less than 300mg 300mg
Sodium	Less than 2,400mg 2,400mg
Total Carbohydrate	300g 375g
Dietary Fiber	25g 30g
Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4	

Fig. 6. Nutrition panels for typical white pan bread and fiber-fortified white pan bread. The added dietary fiber was cross-linked resistant wheat starch.

ning calorimetry (DSC). In the study, cross-linked resistant wheat starch was stored at 4°C for two weeks after cooking at 95°C for 30 min.

Cross-linked resistant starches can deliver many of the health benefits of dietary fiber to consumers without negatively impacting organoleptic and textural properties across a much wider range of food products than is possible with traditional fiber. Applications for cross-linked resistant wheat starch include white breads, nutrition bars, cookies, muffins, waffles, buns, bagels, pastas, noodles, biscuits, snack foods, tortillas, brownies, pizza dough, pretzels, breakfast cereals, crackers, ice cream, and drinks. Formulation examples and nutritional claims are shown in Tables I–III and Figures 6–8.

### Fat Sparring Plus Added Fiber

The functionality of phosphate cross-linked RS4 can be modified by hydrother-

mal treatment to remove crystallinity without losing the individuality of the starch granules (47,56). Commercially available pregelatinized cross-linked resistant starch is produced from wheat starch. Pregelatinized cross-linked resistant wheat starch displays a high dietary fiber content (>75%, dry basis) and low caloric density. When mixed with water, pregelatinized cross-linked resistant starch displays a fat-like texture and is shown to be useful as a fat replacer and fiber enhancer in a wide variety of food products, including cheeses, ice creams, yogurts, brownies, cakes, mashed potatoes, sauces, and salad dressings (39,58). Depending on the food formulation requirement, the functional and nutritional properties of cross-linked resistant starch can be modified further by thermal treatment in the presence of hydrocolloids to increase dietary fiber, emulsion stability, and hot- and cold-water dispersability (57).

**Table I. White pan bread formulated with cross-linked resistant wheat starch. Bread flour was partially replaced with 19% (bakers' percentage) cross-linked resistant wheat starch and 3% vital wheat gluten**

Ingredients	Control (%)	Fiber Fortified (%)
Bread flour	100	78.0
<b>Resistant wheat starch</b>	–	<b>19.0</b>
Vital wheat gluten	–	3.0
Yeast	5.0	5.0
Shortening	5.0	5.0
Salt	2.0	2.0
Sugar	4.0	4.0
Water (variable)	62.0	62.0
Ascorbic acid	75 ppm	75 ppm
Sodium stearoyl lactylate	0.5	0.5

**Table II. Pizza crust formulated with cross-linked resistant wheat starch. Wheat flour was partially replaced with 12.8% (bakers' percentage) cross-linked resistant wheat starch and wheat protein isolate**

Ingredients	Control (%)	Fiber Enhanced (%)
Bread flour	100	85.0
<b>Resistant wheat starch</b>	–	<b>15.0</b>
Salt	2.0	2.0
Water	55.0	55.0
Instant yeast	2.0	2.0
Soybean oil	2.0	2.0
Emplex (SSL)	0.5	0.5

**Table III. A snack cracker formulated with cross-linked resistant wheat starch. The pastry flour was partially replaced with 44.0% (bakers' percentage) cross-linked resistant wheat starch and 6.0% vital wheat gluten**

Ingredients	Control (%)	Fiber Fortified (%)
Pastry flour	100	50.0
<b>Resistant wheat starch</b>	–	<b>44.0</b>
Vital wheat gluten	–	6.0
Sugar, granulated	8.0	8.0
Shortening	12.0	12.0
Nonfat dried milk	1.5	1.5
Salt	1.0	1.0
Sodium bicarbonate	0.5	0.5
Malt, nondiastatic	0.5	0.5
Ammonium bicarbonate	1.0	1.0
Sodium Sulfite	0.04	0.04
Water	7.73	7.73

### Recent Research Update

- Phosphorylated cross-linked wheat starch (RS4), at a calculated intake of 26 g/day by healthy humans, has been shown to be a prebiotic eliciting a high (>300%) and an immediate (2–4 d) increase in gut bifidogenic bacteria (J. Walter, Resistant starch and its impact on gut microbiota composition and metabolism. Book of Abstr. No. 007-02, presented at the 2009 Institute of Food Technologists Annual Meeting and Food Expo, Anaheim, CA, U.S.A.; I. Martinez, P. Duffy, V. Schlegel, and J. Walter. Investigation of the impact of resistant starches on the gastrointestinal microbiota. Unpublished data, 2009).
- In July 2009, the CODEX Alimentarius Commission (CAC) of the FAO/WHO adopted a definition of dietary fiber that reiterated its 2006 definition as stated in this article. However, the 2009 definition increases the minimum degree of polymerization (dp) of carbohydrate polymers from 3 to 10, but defers the final decision on dp to various national authorities. Assay methods for dietary fiber are scheduled for discussion by the commission in fall 2009 ([www.nutraingredients-usa.com](http://www.nutraingredients-usa.com)).

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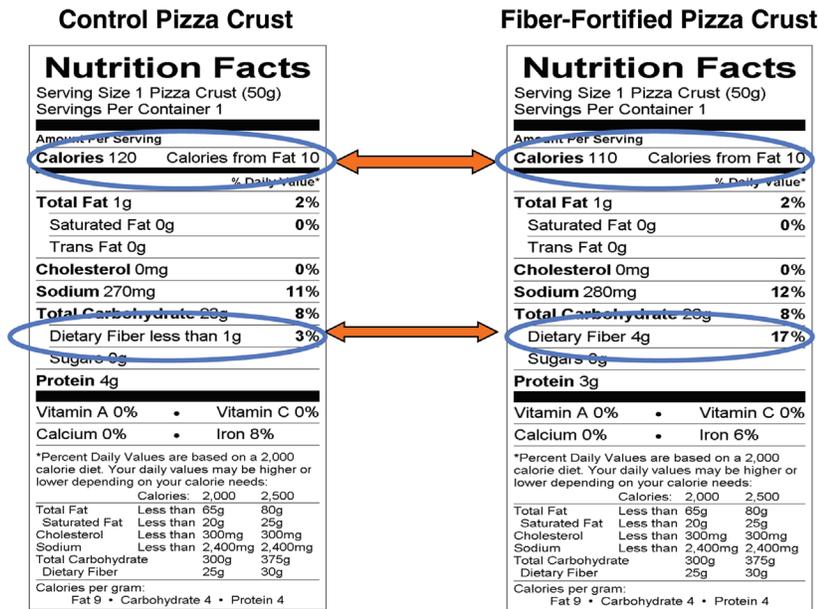


Fig. 7. Nutrition panels for typical pizza crust and fiber-fortified pizza crust. The added dietary fiber was cross-linked resistant wheat starch.

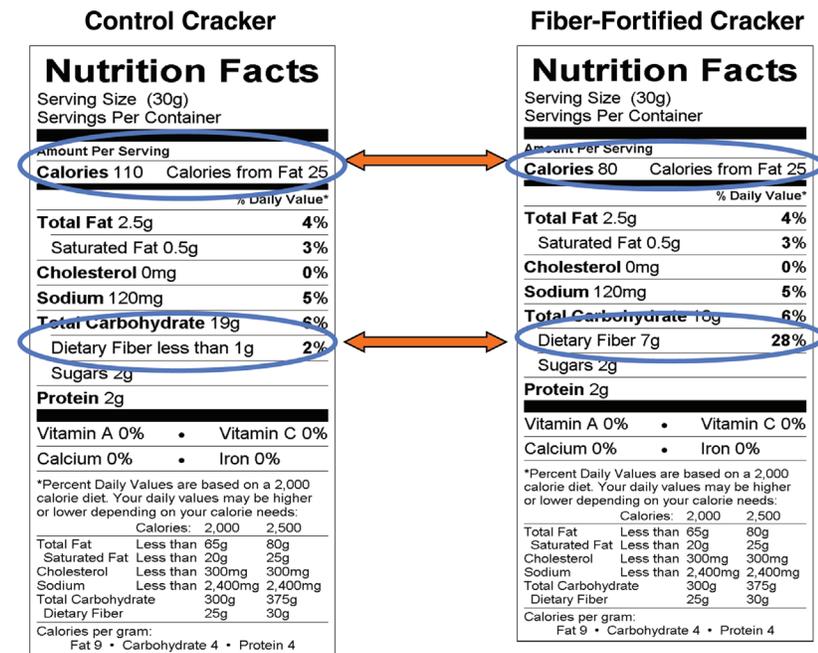


Fig. 8. Nutrition panels for a typical snack cracker and a fiber-fortified snack cracker. The added dietary fiber was cross-linked resistant wheat starch.

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