Comparison of In Vitro and In Vivo Measures of Resistant Starch in Selected Grain Products

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ABSTRACT

The objectives of this research were to determine and compare resistant starch (RS) in typically consumed grain products using an in vivo animal model and two in vitro methods, and to determine the effect of processing for consumption on RS. Home-prepared cake, cookies, and muffins, and commercially processed white bread, corn cereal, and oat cereal were studied. Mean (± standard deviation, $n = 6$) RS content of these foods, determined using the colectomized rat, was 0.6 ± 0.1% (unprocessed) and 1.1 ± 0.7% (processed). Processing did not change RS in the two cereals. Starch remaining in the fiber residue was not similar to that in the in vivo measure of RS. In an in vitro system, developed to simulate gastrointestinal digestion, varying pancreatin (but not pepsin) treatment for 1–5 hr was required to obtain values comparable to those of the in vivo starch bioavailability. Low bacterial counts (≈20% of rat fecal concentration) in the ileal excreta and a continual monitoring of starch bioavailability from canned peas verified minimal microbial degradation of RS and indicated that the colectomized rat was a suitable model for RS studies. Our analyses suggest traditional grain products may provide 4–6 g of RS/day due to consumption levels.

Until the first description of resistant starch (RS) in 1982 (Englyst et al 1982), the prevailing concept was that starch was completely digested and absorbed (Asp 1995). RS was first defined analytically as that fraction not extracted from the nonstarch polysaccharide component of dietary fiber unless the sample had been first treated with alkali or dimethyl sulfoxide (Englyst et al 1982). Subsequent research, as concisely reviewed by Asp (1995), clarified that the RS measured in that manner was retrograded amylose. In addition, it became apparent that there were several other types of RS. These include physically enclosed starch such as that which occurs in beans, ungelatinized starch granules of the B-type found in green bananas and raw potatoes, and chemically modified or dry-heated food starches (Asp 1995).

Unless certain raw or cooled cooked foods are considered, cooked beans and legumes are the only foods that contain substantial amounts of incompletely digested starch (Hildebrandt and Marlett 1991, Björck and Siljeström 1992, Englyst et al 1992, Muir and O'Dea 1992). However, these are not major foods in the typical North American diet. Rather, bakery products and cereals are consumed in substantially greater quantities than are beans and legumes (Block and Lanza 1987). During analysis of foods for dietary fiber content, we observed that the glucose component of the fiber fractions of some cereals increased with processing (Shinnick et al 1988, Marlett et al 1989). We also analyzed unbaked and home-baked chocolate chip and sugar cookies, yellow cake and pie crust, and found more glucose in the fiber from all of the baked products except the pie crust when compared to the fiber in the corresponding unbaked products (M. Longacre and J. A. Marlett, *unpublished data*). The dietary fiber in the unbaked products was comparable to that provided by the flour, which was also analyzed.

Several laboratories (Björck et al 1986, Westerlund et al 1989, Rabe and Sievert 1992) have reported that bread contains more RS than the dough or native wheat starch, although Rabe and Sievert (1992) reported that no RS, assessed by an analytical procedure, was formed during extrusion cooking of wheat flour under severe conditions or during pasta production. The effects on RS formation of several other processes also have been determined, for example, storage, inclusion of food additives, and repeated cycles of heating and cooling (Pomeranz 1992, Ward et al 1994).

RS is of interest because its presence in a food effectively increases the dietary fiber content and decreases the available energy content. Thus, it is important to know the relationship between analytically determined RS and starch actually assimilated in the small intestine. Our objectives were to: 1) measure RS in typically consumed baked products and cereals; 2) determine the effect on RS formation of processing the product for consumption; and 3) compare in vitro measures of RS with starch bioavailability determined in vivo. Three methods of RS determination were compared: 1) starch not digested in vivo that was recovered in the ileal excreta from colectomized rats; 2) an in vitro measure, as starch not removed during Uppsala dietary fiber analysis; and 3) starch not hydrolyzed during an in vitro simulation of in vivo digestion in the gastrointestinal tract. Six test foods were studied in all three experiments; each was studied in the raw (unprocessed) state and again after processing or baking.

MATERIALS AND METHODS

Baked Products and Cereals

Three home-prepared baked products (cake, cookies, and muffins), one commercially baked product (white wheat bread), and two ready-to-eat (RTE) cereals were studied. Typical household recipes (Table I) were used to prepare the cake, cookies, and muffins with slight modifications in the fat content, so that the fat content of the test diets were within the range of 50–80 g/kg. Spices were not added to the recipes. The two cereals were donated by the Quaker Oats Company (Barrington, IL). The cereals were processed using high-pressure extrusion, and the extrudates were dried using conventional drying processes. The white bread was purchased from a local bakery (Triggs Bakery, Madison, WI); the unbaked and baked products were from the same batch. The cereals were ground to 30 mesh and used as is. The baked cookies and bread were air-dried and blended in a blender (Waring Blendor, model 31BL79, New Hartford, CT). Cake and muffins, which were much higher in moisture content (Table I), and the drained canned peas (Del Monte Inc., San Francisco, CA), were blended with sufficient water to form a uniform homogenate, lyophilized (Virtis Freezemobile, Gardiner, NY) and ground with a mortar and pestle.

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Colectomized Rats

Colectomies were performed as previously described (Hildebrandt and Marlett 1991) on male rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 260–340 g using a surgical procedure described by Lambert (1965). Postoperative care and provision of fluids, electrolytes, and nutrients were as described (Hildebrandt and Marlett 1991). We have shown previously that after the animals reach preoperative body weight (day 6–11 after surgery), weight gain is comparable to that of sham operated control animals (Hildebrandt and Marlett 1991). Supplemental vitamin K (menadione sodium bisulfite, Sigma Chemical Co., St. Louis, MO) was provided in the water (500 μg/L) to compensate for the absence of the microbially synthesized vitamin normally absorbed from the colon. Ileal excreta was a soft formed consistency by day 2–5 after surgery. Animals were individually housed in wire bottom cages in a facility that was maintained at a temperature of 70°F, 70% rh, and a light cycle in which 8:00 AM to 8:00 PM was the dark period. Body weight and food intake were measured daily for two weeks after surgery and then three times weekly thereafter.

Bacterial counts in ileal excreta were monitored to determine that the distal ileum did not become colonized like the typical colon. Ileal excreta was collected from each rat two weeks after surgery and approximately every four weeks thereafter until the end of the feeding experiments for the bacterial count determinations as previously described (Cabotaje et al 1990). As further precautions, the animal model was not used if it had been surgically modified for longer than four months, and for each test food studied, one group of control rats was fed diet containing canned peas. Any reduction in the starch recovered in ileal excreta of these control groups would be an indication of colonization of the distal part of the ileum. The surgical procedure and the subsequent feeding experiments were approved by the College of Agricultural and Life Sciences Research Animal Resources Committee, University of Wisconsin-Madison.

TABLE I
Formulas of Home-Prepared Baked Products[a]

	Amount (g)		
	Cake	Cookies	Muffins
Flour, all-purpose	330	621	360
Margarine	110	114	48
Sugar	335	202	96
Eggs	92	55	55
Salt	5.6	2.8	3
Baking soda	...	7	8
Baking powder	7	...	...
Vanilla	7	...	...
Vinegar	...	18	...
Milk, skim	305	...	...
Water	...	118	339

[a] Yields: 8" round cake (double layer); 96 cookies; 24 muffins.

Experimental Diets

Diets were designed to be similar to the macronutrient and micronutrient composition of the AIN-76A purified diet (American Institute of Nutrition 1980). Existing composition data for the test foods were used for diet formulation (Table II). These included food composition tables, analyses from the manufacturer (for the two cereals), and starch data we previously obtained by analysis of similar foods. Except for starch, macronutrient contributions by the home-prepared foods were calculated by summing the amounts contributed by the ingredients (USDA Handbook 8, 1978-1987). Sucrose was by difference; in our experience, these calculations for protein and fat agree with analytical data. Macronutrient composition of the bread also was from Agriculture Handbook 8. Subsequently, test foods were analyzed for dietary fiber and starch content (Table III).

Peas were incorporated into a purified diet (American Institute of Nutrition 1980) at a level to contain 10% dietary fiber as previously done (Hildebrandt and Marlett 1991) (Table II). Pea fiber (5%) was substituted for the cellulose normally in the AIN-76A diet; the other 5% was added by dilution, so that when the animal consumed diet to meet energy needs, the ratios of micronutrients to energy were not changed (Shinnick et al 1988).

Because the macronutrient composition of the test products varied, the amount of each incorporated into the purified diet was arbitrarily set at about 600 g/kg (air-dried and lyophilized weight). This level allowed for at least 100 g/kg of high quality protein (casein) in the diet, but it was not so high that the fiber contributed by the test product exceeded ≈50 g/kg. Micronutrients and purified sources of macronutrients were added as needed to make the composition of the diets similar to that of the AIN-76A formula (Table II). Cellulose was added to the diets to bring the level of dietary fiber to 5%. The oat cereal products were higher in fiber content than the other test products so that when ≈600 g of product was incorporated, fiber concentration of the final diet was 53 and 52 g/kg for the unprocessed and processed products, respectively. A nonabsorbable marker, chromic oxide (0.6%) (Fisher Scientific, Pittsburgh, PA), was added to all diets so that the amount of starch in ileal excreta could be related to the amount of starch consumed (Hildebrandt and Marlett 1991).

Feeding Experiments

Twelve colectomized rats, weighing ≈350–450 g, were randomly divided into three groups of four animals each. One group was fed a diet containing the unprocessed product, another group was fed the processed product, and the third group was fed a purified diet containing canned peas. Each test diet was fed ad libitum for 12 days. One group of 12 rats was used in three

TABLE II
Composition (g/kg[a]) of Rat Diets[b]

		Cake		Cookies		Muffins		Bread		Corn Cereal		Oat Cereal	
Ingredient[c]	Peas	Unbk	Bk	Unbk	Bk	Unbk	Bk	Unbk	Bk	Unpro	Pro	Unpro	Pro
Test food	422	600	600	600	600	590	592	590	590	595	590	585	585
Casein	102	153	154	150	150	141	141	109	109	140	142	115	114
Corn oil	39	...	...	...	...	...	...	20	20	22	23	12	12
Cornstarch	285	155	158	158	158	152	152	142	135	52	70	193	194
Sucrose	104	...	...	...	...	28	28	60	69	120	108	45	45
Cellulose	...	42	38	42	42	39	37	29	27	21	17	0	0
Others[c]	48	50	50	50	50	50	50	50	50	50	50	50	50
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

[a] As is weight, except for test food which is lyophilized weight.
[b] Abbreviations: unbk = unbaked, bk = baked, unpro = unprocessed, and pro = processed.
[c] All diets contained an additional 50 g (as g/kg): 10 vitamin mix (AIN-76A), 35 mineral mix (AIN-76), 3 methionine and 2 choline chloride. All diet ingredients, except for the test foods, were purchased from Teklad Test Diets, Madison, WI.

experiments to study the three home-prepared products. A second group of 12 colectomized rats was used in the other three experiments to evaluate the two commercial cereals and the commercially baked white bread. Ileal excreta was collected for chromium and starch analysis at frequent intervals (every 2 hr) from 8:00 a.m. to 4:00 p.m. during days 8–12 of each experiment to minimize the time for continued fermentation after excretion. Excreta from each rat was frozen (–5°C) immediately after collection and pooled for five days. Ileal excreta was lyophilized, ground with a mortar and pestle, and passed through a 600-μm screen to remove hair. Excreta passed between 4:00 p.m. and 8:00 a.m. the following day were collected at 8:00 a.m., processed as described, and weighed but not analyzed, so that daily ileal excreta output could be measured.

Starch Bioavailability in Colectomized Rat

Triplicate aliquots (25–50 mg) of each test food and pooled ileal excreta from each rat were analyzed for starch content after simple sugar extraction by refluxing with 80% ethanol (AOAC 1980). Starch in the ethanol-insoluble residue was enzymatically hydrolyzed after residue aliquots had been autoclaved (1.5 hr, 121°C) and the resulting glucose was quantitated using glucose oxidase (AOAC 1980). Chromium was determined in triplicate aliquots of diet and duplicate aliquots of ileal excreta (50 mg) by atomic absorption spectrophotometry (Guncaga et al 1974). The amount of diet (g) represented by the five days of ileal excreta was calculated as: chromium in ileal excreta (mg) divided by the analyzed concentration of chromium in the diet (mg/g). The percentage of starch from the test food not digested by the rat was calculated as: (starch [mg] in 5 days of ileal excreta divided by the amount of test starch [mg] ingested in 5 days) × 100. The amount of test starch ingested was calculated from its concentration in the test diet, which was determined by analysis (Table III). This concentration was multiplied by the amount of diet consumed. Digestibility of the purified cornstarch is complete in the colectomized rat (Hildebrandt and Marlett 1991) and thus, was not included in the calculation.

In Vitro Starch Digestibility Determined by Fiber Analysis

The dietary fiber content and composition in duplicate aliquots (≈10 g, dwb) of the test foods were determined as the sum of the fiber components in the soluble and insoluble fractions using a modification (Shinnick et al 1988) of the enzymatic-chemical method A of Theander and Westerlund (1986), which has been renamed the Uppsala procedure. Triplicate aliquots (≈15–25 mg) of the soluble and insoluble fractions were autoclaved and assayed for starch content using the enzymatic-colorimetric procedure described above.

TABLE III
Contribution of Starch and Dietary Fiber from Test Foods (g/kg of diet) to Test Diets[a]

	Starch		Dietary Fiber	
Food	**Calc.**	**Anal.**	**Calc.**	**Anal.**
Cake				
Unbaked	195	170	8	8
Baked	194	160	12	10
Cookie				
Unbaked	330	270	8	11
Baked	330	300	8	12
Muffin				
Unbaked	346	280	11	13
Baked	346	290	13	17
Bread				
Unbaked	354	350	21	18
Baked	361	360	23	22
Corn cereal				
Unprocessed	446	420	29	26
Processed	428	410	33	23
Oat cereal				
Unprocessed	301	360	53	53
Processed	300	340	57	52
Peas	196	85	100	101

[a] Calculated data were from previous analyses of foods similar to test foods, except for cereals data which were supplied by the manufacturer.

In Vitro Starch Digestibility Assay

All test foods, except for the peas, were defatted before enzymatic starch digestion using petroleum ether (25 ml/g × 3, 15 min, ambient temperature). Fat-extracted samples (200 mg) were heated (3 min) to 75°C with water (9 ml) to inactivate endogenous enzymes in the unprocessed products and cooled to room temperature before the pH was adjusted to 1.5–1.7 using 1*N* HCl. Pepsin (5 mg) (E.C. 3.4.23.1, catalogue number P-6887, Sigma) was added and the samples were incubated (60 min, 37°C) with shaking. Samples were neutralized with 1*M* NaOH; phosphate buffer (pH 6.9, 10 ml of 0.1*M* phosphate) and pancreatin (40 mg) (catalogue number P-7545, Sigma) were added and the samples were incubated (37°C) again, this time for 1–6 hr, with shaking. Enzyme activity was terminated by rapidly heating the sample to boiling (Faulks and Bailey 1990). Undigested starch was determined in the pellet obtained by centrifugation (1,520 g, 10 min); the pellet was washed twice with 80% ethanol (5 ml). Starch was measured in duplicate in each of the three in vitro assays for each food after autoclaving, as described above.

Statistical Analysis

Analysis of variance (ANOVA) was used to test for the differences among the six groups. Fisher's protected least significant difference (LSD) procedure was used to make specific comparisons between groups. A difference of $P < 0.05$ was considered statistically significant.

TABLE IV
Daily Food Intake and Ileal Excreta (g/24 hr) of Colectomized Rats[a]

		Ileal Excreta	
Diet	**Food Intake**	**Wet**	**Dry**
Cake			
Unbaked	24.3 ± 0.3	5.4 ± 0.2	2.1 ± 0.1
Baked	22.8 ± 1.3	5.3 ± 0.6	2.0 ± 0.2
Peas	27.0 ± 1.4	18.7 ± 1.1a	4.8 ± 0.3
Cookies			
Unbaked	23.4 ± 0.6c	5.6 ± 0.1	2.2 ± 0.1
Baked	26.3 ± 1.7d	5.6 ± 0.6	2.2 ± 0.3
Peas	26.6 ± 1.1	22.5 ± 3.0b	5.2 ± 0.4
Muffin			
Unbaked	22.4 ± 2.6	5.1 ± 0.7	2.1 ± 0.2
Baked	22.1 ± 0.8	5.5 ± 0.7	2.1 ± 0.1
Peas	22.7 ± 1.8	20.4 ± 1.9ab	4.8 ± 0.6
Bread			
Unbaked	21.6 ± 1.5	5.3 ± 0.7	2.0 ± 0.3
Baked	23.4 ± 1.2	6.3 ± 0.6	2.2 ± 0.1
Peas*	23.0 ± 1.1	22.5 ± 2.2b	5.0 ± 0.3
Corn Cereal			
Unprocessed	23.4 ± 1.0	7.8 ± 0.5c	2.6 ± 0.2d
Processed	24.1 ± 1.9	6.3 ± 0.4d	2.1 ± 0.1c
Peas	24.2 ± 1.5	20.4 ± 1.8ab	4.9 ± 0.2
Oat Cereal			
Unprocessed	22.4 ± 1.0	8.8 ± 0.4	2.9 ± 0.1
Processed	22.6 ± 1.2	10.1 ± 1.2	2.8 ± 0.2
Peas	23.3 ± 1.1	18.7 ± 1.4a	4.7 ± 0.4

[a] Mean ± standard deviation, n = four rats per group; except *, n = 3. Food intake was measured throughout each 12 day experiment, ileal excreta output was measured during the time it was collected for analysis (day 8—12). Significant differences ($P < 0.05$) in food intake or ileal excreta output between rats consuming unprocessed vs. processed form of same test food and among all groups of rats consuming the control pea diets are indicated by different letters (analysis of variance and least significant difference).

RESULTS AND DISCUSSION

Colectomized Rats

All diets were well accepted by the rats. There were no significant differences between food intake of each paired group of rats fed processed vs. the comparable unprocessed product, except for the rats fed cookies (Table IV). Processing altered the daily output of ileal excreta during only one food test; the wet and dry weights decreased when the processed corn cereal was consumed, compared to the unprocessed product (Table IV). Differences in food intake and output of ileal excreta among the groups of rats fed different test foods and among groups fed the control diet containing canned peas were observed as expected as they had different body weights during each feeding experiment (statistical significance not shown).

Data from the first group of colectomized rats fed the home-prepared products indicated that bacterial counts remained low and were about 20% of the counts usually measured in rat feces, 1×10^{11}/g of dry feces (Cabotaje et al 1990) (Table V). Bacterial counts in ileal excreta from the second group of rats that were fed the cereals and commercial bread were comparable to those of the first group over the postoperative period (weeks 2–12) used for this study (data not shown). Some bacteria are normally found in the intact distal small bowel (Rowland et al 1986).

The mean (± standard deviation) percent of undigested pea starch among the six groups of control rats fed canned peas was 35.3 ± 2.3% (range 32.1–39.1%) and did not change with time after colectomy surgery. These results are similar to those of the processed pea starch (30%) that was recovered in the feces of antibiotic-treated young rats (Björck and Siljeström 1992). We concluded from these two serial measurements that little microbial degradation of RS occurred in the terminal ileum in the colectomized rat during the course of the experiments.

TABLE V
Bacterial Counts in Ileal Excreta from Colectomized Rats[a]

Postoperative Week	Counts (10^{10}/g of dry wt)
2	1.6 ± 0.5[a]
6	2.8 ± 1.2
10	2.4 ± 0.9
13	3.6 ± 0.6
16	1.8 ± 0.8*
18	1.5 ± 0.7

[a] Mean ±standard deviation, n = 12 rats (except *, n = 11).

Calculated vs. Analyzed Starch and Dietary Fiber Contents of Test Foods

Because the test foods contained different amounts of ingredients, they provided different amounts of starch when they were incorporated into a nutritionally adequate rat diet at a level of ≈600 g/kg (Table III). The analyzed starch contents of all test foods except for the oat cereal were lower than the calculated data. Although this slightly lowered the total carbohydrate content of the diet, it had no effect on its nutritional quality.

As expected, the analyzed dietary fiber values were slightly different from those used to formulate the diets. The actual dietary fiber contents of the diets were within 5 g of the formulated 50g/kg, except for the processed corn cereal. The calculated value for the corn cereal, obtained by the AOAC method, was greater (3.3%) than what was determined by the Uppsala method (2.3%). We previously reported different dietary fiber contents by these two methods for grain products and proposed that the short amylase treatments in the AOAC method do not always completely hydrolyze starch (Marlett and Vollendorf 1994). Because of this difference in analytical methods, the processed corn cereal diet actually contained 4% dietary fiber instead of the expected 5%.

In Vivo Starch Digestibility

Less than 1% of the test food starch in the six unprocessed products was recovered in the ileal excreta (Table VI). Baking the three home-prepared products or the commercial bread significantly decreased ($P < 0.05$) the digestibility of starch, although starch digestibility remained high in all of the test foods (Table

TABLE VI
In Vivo and In Vitro Measures of Starch Bioavailability from Grain Products (% Starch Undigested)[a]

			In Vitro Incubation (hr)[d]				
	In Vivo[b]	Dietary Fiber Analysis[c]	1	2	3	4	5
Peas, canned	35.3 ± 2.3a	15.3b	41.7 ± 0.0c	38.3 ± 0.8ac	···	38.6 ± 0.2c	37.3 ± 0.1a[e]
Cake							
Unbaked	0.8 ± 0.2a	Trace b[f]	1.0 ± 0.1a	0.3 ± 0.1b	0.3 ± 0.1b	0.3 ± 0.1b	
Baked	2.2 ± 0.2a	1.1b	2.4 ± 0.2a	1.5 ± 0.1c	1.5 ± 0.0c	1.4 ± 0.0c	
Cookies							
Unbaked	0.6 ± 0.1a	Trace b	2.9 ± 0.2c	1.4 ± 0.1d	0.5 ± 0.0a	0.3 ± 0.0b	
Baked	0.9 ± 0.1a	Trace b	3.6 ± 0.3c	2.3 ± 0.2d	1.8 ± 0.2e	1.3 ± 0.0f	1.1 ± 0.1af
Muffins							
Unbaked	0.5 ± 0.0a	Trace b	2.6 ± 0.2c	1.4 ± 0.1d	0.9 ± 0.1e	0.6 ± 0.1a	
Baked	1.5 ± 0.0a	1.2b	3.6 ± 0.1c	2.4 ± 0.2d	1.9 ± 0.1e	1.4 ± 0.1a	
Bread							
Unbaked	0.6 ± 0.1a	0.3b	3.0 ± 0.3c	1.6 ± 0.1d	1.0 ± 0.1e	0.6 ± 0.1a	
Baked	1.4 ± 0.0a	1.0b	6.9 ± 0.2c	3.9 ± 0.1d	1.8 ± 0.2e	1.4 ± 0.1a	
Corn cereal							
Unprocessed	0.4 ± 0.0a	Trace b	1.5 ± 0.1c	1.0 ± 0.1d	0.6 ± 0.1e	0.4 ± 0.0a	
Processed	0.3 ± 0.1a	0.3a	6.0 ± 0.3b	3.5 ± 0.2c	2.1 ± 0.0d	1.3 ± 0.2e	0.5 ± 0.1a
Oat cereal							
Unprocessed	0.5 ± 0.0a	0b	2.2 ± 0.2c	1.0 ± 0.0d	0.4 ± 0.0a	Trace b	
Processed	0.4 ± 0.0a	0b	2.9 ± 0.2c	1.2 ± 0.1d	0.5 ± 0.2a	Trace b	

[a] Data in same row with different superscripts are significantly different ($P < 0.05$). Data are for oven-dried food.
[b] Mean ± standard deviation measured in ileal excreta from four colectomized rats. Mean ± standard deviation for peas is from the six groups of four rats that were the control groups for each test food experiment.
[c] Mean of two analyses.
[d] Mean ± standard deviation of three replicates, except for peas, where n = 2. Time refers to duration of incubation with pancreatin which was preceded by a pepsin incubation (1 hr, 37°C, pH 1.5—1.7), and except for peas, fat extraction using petroleum ether.
[e] Value for 6-hr incubation.
[f] Trace < 0.3%

VI). Even in the baked cake, which had the lowest digestibility of the test foods that were studied, only 2.2% of the test food starch was recovered in ileal excreta.

Other in vivo studies of starch bioavailability have used primarily human ileostomates (Englyst and Cummings 1985, Schweizer et al 1990, Andersson 1992, Muir and O'Dea 1993) or antibiotic-treated young rats (Asp et al 1992, Björck et al 1986). Although comparisons among data from different studies are confounded by use of different methods, in vivo bread starch digestibilities of ≥98% reported by others (Englyst and Cummings 1985, Björck et al 1986, Andersson 1992) are similar to what we observed using the colectomized rat. We extended these observations by showing that typical household baking conditions, which are similar to those in commercial bakeries, had only small effects on in vivo starch bioavailability.

Oat bran was previously reported to be completely digested (Englyst and Cummings 1985), and our study suggests that at least one commercial process used to manufacture a RTE oat cereal did not decrease the bioavailability of starch in oat bran. Processing corn into a RTE flaked cereal renders 3–5% of the starch as RS (Schweizer et al 1990, Muir and O'Dea 1993), whereas the commercial process used to prepare the corn RTE cereal we studied had no effect on starch bioavailability. In contrast, high-amylose corn hydrothermically treated (Molis et al 1992) or processed into a Latin American bread (*arepas*) (Granfeldt et al 1993) was poorly digested in the small intestine of intact human subjects (Molis et al 1992) or antibiotic-treated rats (Granfeldt et al 1993). These differences in corn starch bioavailability are likely caused by differences in the corn variety, processing conditions, and other ingredients in the product.

In Vitro Measures of RS

The starch not enzymatically extracted during dietary fiber analysis but susceptible to attack after autoclaving was not a good indicator of the starch bioavailability determined in the colectomized rat (Table VI). Hydrolytic steps used in the fiber analysis procedure more effectively extracted starch from all test foods, except the processed corn cereal from which the analytical and in vivo methods both measured only very small amounts of undigested starch (0.3%). Such a result is not unexpected. Dietary fiber methods are designed to extract starch so that it does not inflate the dietary fiber value. Achieving agreement between in vivo starch digestibility and amount of starch not extracted during dietary fiber analysis appears to depend on the method of fiber analysis, the method of extracting the RS, the enzymes used, conditions of enzymatic hydrolysis, and the foodstuff (Faulks and Bailey 1990, Asp et al 1992, Björck and Siljeström 1992, Faisant et al 1993, Granfeldt et al 1993).

No single set of experimental conditions designed to mimic in vivo digestion uniformly predicted starch bioavailability measured in the colectomized rat (Table VI). The duration of in vitro digestion with pancreatin necessary to mimic in vivo starch bioavailability ranged from 1–5 hr for the three home-prepared products, even though all were baked using typical household oven conditions. The pancreatin incubation step also ranged from 3–4 hr for the commercially processed samples. Processing affected the duration of this incubation for two samples (cookies and corn cereal); both had to be incubated longer than their comparable unprocessed product to achieve a level of undigested starch similar to that determined in vivo. In contrast, preliminary experiments indicated that varying the duration of pepsin treatment (1–5 hr) had no effect on the results.

Two in vitro methods have been proposed to measure all physiologically relevant RS (Asp 1995). One method (Muir and O'Dea 1992, 1993) mimics in vivo digestion by treating a sample with pepsin after it has been chewed; subsequent incubation with amylases for 15 hr is needed to achieve a level of starch digestibility comparable to what is measured in human ileostomates (Muir and O'Dea 1993). The procedure we developed is similar to the one developed by Muir and O'Dea (1992, 1993) in that it also used an initial pepsin treatment; however, subsequent amylase incubation needed to attain an extent of starch digestion comparable to what was measured in vivo was much shorter in our procedure. The difference between the two methods in the time required for starch digestion may be due to our use of a homogenized instead of a masticated sample, an initial fat extraction, or pancreatin, a crude enzyme preparation that has both proteolytic and amylolytic activities. The process of gastric mixing is forceful and effective, particularly in combination with acid and pepsin, and efforts to mimic chewing before in vitro procedures (Englyst et al 1992, Muir and O'Dea 1992) without mimicking gastric action also may be part of the reason for the differences.

The second in vitro procedure developed to measure physiologically relevant RS does not employ a pepsin treatment and includes a variety of approaches to simulate chewing (Englyst et al 1992). This method yielded RS values for white bread, rice (Englyst et al 1992), and peas (Englyst and Kingman 1990) comparable to what we measured in vivo in this study for peas and white bread or what we measured previously for rice (Hildebrandt and Marlett 1991). However, their method measured 25% (dwb) RS in sweet corn, whereas we found sweet corn starch to be highly digestible in the colectomized rat (Hildebrandt and Marlett 1991). Other in vitro measures of RS in bread are similar to what we measured (Björck et al 1986, Westerlund et al 1989, Holm and Björck 1992).

Conclusions

In vivo digestibility of starch in a selected number of commonly consumed grain products was high (≥98%) and was decreased, although marginally so, by typical processing for consumption. Cereal and grain products can provide significant undigested starch to the large intestine, however, by virtue of the amounts consumed. For example, a realistic intake of the foods we studied would provide 4.1 g/day of in vivo RS, whereas one serving of canned peas provides 5 g/day (Table VII).

In vitro measurement of in vivo starch bioavailability still varies with the method and test food (Hildebrandt and Marlett 1991, Champ 1992, Englyst et al 1992, Muir and O'Dea 1992, 1993). Efforts to mimic the dynamic processes that comprise digestion in the upper gut are difficult, and none of the analytical measures have been validated by in vivo measurements using a wide spectrum of foods or complex meals. Arguments can be presented for and against animal vs. human models for validation of RS analysis. Although data are limited, the colectomized rat appears to digest starch to the same extent as human ileostomates. We chose to develop the colectomized rat because ileostomies in humans have become uncommon surgical procedures in the United States; the animal model is not only more accessible but is also less costly and much easier to use than human ileostomates.

TABLE VII
Resistant Starch (RS) in Single Serving of Grain Products and Canned Peas[a]

	Serving Size		RS
Food	Household	(g)	(g/serving)
Cake	1 wedge	95	1.7
Cookies	5	40	0.3
Muffin	1	40	0.4
Bread, white	2 slices	52	0.5
Cornflakes	1 bowl	29	1.2
Peas, canned	½ cup	80	5.0

[a] Fresh weight.

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