

Resistant Starch and Total Dietary Fiber Content of Oatrim Muffins with Different Levels of Amylose, Amylopectin, and β -Glucan

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ABSTRACT

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Nine types of muffins made with three levels of β -glucan and three amylose-amylopectin ratios were prepared at the Beltsville Human Nutrition Research Center, United States Department of Agriculture. They were fed to human subjects to study effects of starch composition and dietary fiber content on the carbohydrate and lipid metabolism in normal and overweight women. The main objective of this study was to determine resistant starch (RS) and total dietary fiber (TDF) content of the muffins 1) using AACC Approved Method 32-07 and AOAC method 991.43, incorporating a pretreatment step with dimethyl sulfoxide (DMSO) before

enzyme incubation, 2) with pretreatment at 100 and 121°C before incubation with amyloglucosidase, and 3) using samples chewed by human subjects before incubation with pancreatin and amyloglucosidase. For method 1, on an as-eaten basis, TDF content was 2.81 to 9.64 g/100 g for samples without DMSO pretreatment and 1.66 to 4.06 g/100 g with DMSO pretreatment. RS content was 0.30 to 11.18 g/100 g for methods 1 and 2, respectively. Methods 2 and 3 had the best correlation for all muffins tested ($r^2 = 0.97$).

It had been reported, as early as 1977, that undigested starch was recovered from feces of both humans and mice after ingesting baked muffins prepared from amylo maize starch but not from ordinary maize starch (Wolf et al 1977). Since then, *in vitro* methods for the determination of resistant starch (RS) have been developed and modified by various analysts (Englyst et al 1982; Berry 1986; Muir and O'Dea 1992; Saura-Calixton et al 1993; and Faisant et al 1995). An excellent review has been compiled by Asp et al (1996). Recently, Sambucetti and Zuleta (1996) measured total dietary fiber (TDF) using prolonged incubation time with amyloglucosidase and determined the recoverable starch in the fiber residues. They concluded that for TDF results in starchy foods obtained from enzymatic-gravimetric methods, a correction should be made for starch as well as for protein and ash. Ranhotra et al (1999) analyzed 30 grain-based foods for RS by determining the total dietary fiber content according to the AACC Approved Method 32-07 and AOAC method 991.43 with or without dimethyl sulfoxide (DMSO) pretreatment of the samples before enzyme incubation (AACC 2000). Åkerberg et al (1998) modified a method that includes chewing before incubation with proteolytic and amylolytic enzymes at $\leq 40^\circ\text{C}$. This method is capable of including the major forms of RS likely to be in foods, including physically encapsulated starch. We have shown previously that RS content of cooked dried beans can be determined using different autoclave temperatures before incubation with amyloglucosidase (Li and Zhao 1997). As mentioned earlier, there are other methods given in the literature for measuring RS in different types of food; however, no officially approved method exists at the present time. One of the objectives of this study is to determine starch and total dietary fiber content of nine types of muffins made from Oatrim with varying levels of β -glucan and corn starch containing different amounts of amylose and amylopectin using an official TDF method with some modification. We also compared the values of RS obtained from three methods using dif-

ferent enzymes and experimental conditions. Test muffins from the same batches were fed to human subjects to study effects of starch composition and fiber content on carbohydrate and lipid metabolism in normal and overweight women. This is accomplished by evaluating the combined effects of slowly digested or RS and fiber sources on glucose and insulin response and by determining whether the action of fiber and RS are independent, additive or inhibitory in improving glycemic indices to a meal tolerance. Data from this study will be used to evaluate the results from the human study and the final report will be published elsewhere. Previous controlled human feeding studies conducted at the Beltsville Human Nutrition Research Center (BHNRC) found improved glucose and insulin responses after consumption of soluble oat fiber, high amylose cornstarch, and high fiber diets (Behall and Howe 1995; Behall et al 1988, 1989; Hallfrisch et al 1995).

MATERIALS AND METHODS

Materials

Nine types of muffin varying in amylose, amylopectin, and β -glucan content were prepared in the Human Study Facility at BHNRC, USDA, Beltsville, MD. Three groups of muffins were made with standard cornstarch, a high-amylose cornstarch, or a blend of 1:1 standard cornstarch and high-amylose cornstarch. Oatrim containing A) 1% β -glucan, B) 10% β -glucan, or a combination of A and B (5:1) was added to each of the starches to give three levels of β -glucan in each group of muffins. (Table I)

Standard cornstarch (30% amylose and 70% amylopectin) and high-amylose cornstarch (70% amylose and 30% amylopectin) were obtained from American Maize Product Co. (Hammond, IN). Oat fiber extract with either 1% (low) or 10% (high) soluble β -glucans (by weight) was used as the soluble fiber source. Both oat fiber extracts (low and high) were provided by Quaker Oats Co. (St. Louis) and ConAgra Inc. (Omaha, NE). Starches were dried before weighing the dry ingredients for each batch of muffins. Starch and Oatrim were weighed and maintained separately until muffins were made. Identical sets of the other dry ingredients were weighed in sufficient number to be blended with starch and Oatrim. On the day the muffins were made, liquid ingredients were weighed and added to the dry ingredients. Muffins were mixed to incorporate the liquid into the dry ingredients. Batter was weighed before baking at 350°F for 25 min. Muffins were weighed again after they cooled and before they were frozen for storage.

Sample Preparation

Three identical types of frozen muffins were freeze-dried individually to determine the moisture content as eaten, then ground in

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a coffee grinder (type 203; Robert Krups, North America, Englewood Cliff, NJ), mixed thoroughly in heavy-duty Ziplock bags, and stored in a desiccator until analysis.

Analysis

Method 1. AACC Approved Method 32-07 and AOAC Method 991.43 (Lee et al 1992) were modified. Each beaker containing ≈1 g of muffin sample was weighed on a top-loader balance; 5 mL of DMSO was added to two of the beakers, which were covered with aluminum foil and then sonicated, heated for 30 sec in an oven at 100°C, sonicated once more, and heated for an additional 30 min. Two other beakers containing sample and one blank beaker with DMSO were set aside.

MES-TRIS buffer (pH 8.2, 40 mL) was added to all beakers and the samples were incubated sequentially with three enzymes (α -amylase, protease, and amyloglucosidase from a total dietary fiber kit [Sigma]) as in the original method. Beakers and contents were weighed, the weight of total enzyme digest was calculated, and four times the weight of 95% ethanol was added to each beaker. The contents were vigorously stirred and sonicated, then

allowed to stand for 1 hr at room temperature. Beakers containing the dilute ethanolic mixture were weighed again. Without disturbing the precipitates, two aliquots (0.5 mL) were removed from each beaker and dispensed into tared vials and weighed. The contents in the vials were dried under vacuum and derivatized for glucose determination by gas chromatography (Li 1996). Starch content was calculated as glucose \times 0.9. Mixtures in the beakers were filtered through tared fritted crucibles (containing 0.5 g of Celite filter aid) to isolate total dietary fiber residues (DFR). The weight of residue minus the weight of crude protein and ash in the residue is the total dietary fiber. Resistant starch for method 1A was estimated as the difference between the starch content of a sample treated with DMSO and one without DMSO; in method 1B, it was estimated as the difference between the TDF content of the test samples with and without DMSO.

Method 2. Duplicate portions (0.5 g) of ground and freeze-dried muffin were weighed into 50-mL tubes coated with fluorinated ethylene propylene (FEP) and suspended in 25 mL of deionized water. For initial starch gelatinization, one set was heated for 1 hr at 100°C in an oven and another set at 121°C in an autoclave.

TABLE I
Sample Identification

Starch	Oatrim (% β -Glucan)		
	1%	2.5%	10%
30% amylose/70% amylopectin	AP-Low	AP-Mod	AP-High
50% amylose/50% amylopectin	MX-Low	MX-Mod	MX-High
70% amylose/30% amylopectin	AM-Low	AM-Mod	AM-High

TABLE IV
Comparison Between Methods Expressed as Correlation Coefficients

Method	Dry Weight Basis	As-Eaten Basis
1A vs. 2	0.79	0.80
1B vs. 2	0.89	0.90
1A vs. 3	0.89	0.87
1B vs. 3	0.93	0.89
2 vs. 3	0.96	0.97

TABLE II
Starch and Total Dietary Fiber (TDF) Content of Oatrim Muffins (with or without DMSO pretreatment): Method 1^a

Content		AP (g/100 g)			MX (g/100 g)			AM (g/100 g)		
		Low	Mod	High	Low	Mod	High	Low	Mod	High
With DMSO										
Starch	dwb	73.17	72.21	71.49	72.48	71.87	69.89	73.73	69.77	64.03
	as-eaten	49.56	46.39	40.08	48.98	46.42	41.05	50.75	46.42	38.16
TDF	dwb	3.03	3.47	7.25	2.78	2.96	6.91	2.74	3.11	6.51
	as-eaten	2.05	2.23	4.07	1.88	1.91	4.06	1.88	2.07	3.88
Without DMSO										
Starch	dwb	71.47	71.75	69.89	68.35	69.97	61.77	65.58	61.48	54.90
	as-eaten	48.41	46.09	39.19	46.18	45.19	36.28	45.14	40.90	32.72
TDF	dwb	4.76	3.62	8.62	6.43	6.90	11.48	9.80	9.99	16.17
	as-eaten	3.22	2.32	4.83	4.34	4.46	6.74	6.75	6.65	9.64
Difference in										
Starch	dwb	1.70	0.46	1.59	4.13	1.90	8.12	8.15	8.30	9.13
	as-eaten	1.15	0.30	0.89	2.79	1.23	4.77	5.61	5.52	5.44
TDF	dwb	1.74	0.15	1.37	3.65	3.95	4.58	7.07	6.88	9.67
	as-eaten	1.18	0.09	0.77	2.47	2.55	2.69	4.86	4.58	5.76

^a Mean of duplicate analyses. TDF = total dietary fiber, DMSO = dimethyl sulfoxide, AM = amylose, AP = amylopectin, MX = 1:1 mix.

TABLE III
Comparison of Resistant Starch (RS) Content by Three Methods

Muffin ^a	Method (g/100 g of dry weight)				Method (g/100 g, as-eaten)			
	1A ^b	1B ^c	2	3	1A	1B	2	3
AP-low	1.70	1.74	0.45	2.67	1.15	1.18	0.31	1.76
AP-mod	0.46	0.15	1.29	3.11	0.30	0.09	0.83	1.86
AP-high	1.59	1.37	0.80	4.25	0.89	0.77	0.45	2.14
MX-low	4.13	3.65	7.12	11.36	2.79	2.47	4.84	7.51
MX-mod	1.90	3.95	10.89	10.28	1.23	2.55	7.08	6.28
MX-high	8.12	4.58	6.68	11.25	4.77	2.69	3.94	6.22
AM-low	8.15	7.07	14.97	16.28	5.61	4.86	10.33	10.60
AM-mod	8.30	6.88	16.68	17.28	5.52	4.58	11.18	10.90
AM-high	9.13	9.67	13.45	15.96	5.44	5.76	8.07	8.00

^a TDF = total dietary fiber, DMSO = dimethyl sulfoxide, AM = amylose, AP = amylopectin, MX = 1:1 mix.

^b Starch with DMSO – starch without DMSO.

^c TDF without DMSO – TDF with DMSO.

After cooling all the tubes to $\approx 60^{\circ}\text{C}$, a solution of amyloglucosidase (Boehringer-Mannheim No. 208-469) in 1 mL of 4M acetate buffer (pH 4.8) and 1 mL of deionized water was added to each tube, and the contents were mixed and incubated at 55°C for 2 hr with the tubes lying on their sides and rolled occasionally. The tubes were centrifuged at 2,000 rpm for 10 min, and supernatants were decanted into a 50-mL volumetric flask, then diluted with deionized water. The diluted solution (1 mL) was further diluted to 100 mL with water. Approximately 2 mL of the final diluted solution was filtered through a membrane to remove any particulates. Filtrate (1 mL) was mixed with 100 μL of an internal standard (rhamnose, 50 $\mu\text{g}/\text{mL}$) and analyzed for glucose by high-performance anion-exchange chromatography using a pulsed amperometric detector (Li 1998). Starch was calculated as glucose $\times 0.9$. Resistant starch was estimated as the difference between the starch content of a sample heated at 100°C and one autoclaved at 121°C .

Method 3. Total starch was analyzed in milled test products by using sequential incubations with α -amylase and amyloglucosidase after solubilization in 2M KOH (Siljeström et al 1988). Separation of total starch into available starch and RS was performed according to Åkerberg et al (1998). An amount of muffin, corresponding to 1 g of total starch, was chewed 15 times for ≈ 15 sec (six subjects participated in the study). After chewing, the muffin was expectorated into a beaker containing distilled water and pepsin. The subjects rinsed their mouths with distilled water for 60 sec and expectorated the rinsing solution into the beaker. After adjusting to pH 1.5 with HCl, the samples were incubated at 37°C for 30 min. A sodium acetate buffer was added and adjusted to pH 5.0 with NaOH. Pancreatin, amyloglucosidase, isopropanol, Ca, and Mg salts were added to the beakers equipped with a magnet (floating stir bar). The beakers were covered with parafilm, put into place with rubber band, and incubated for 16 hr at 40°C with constant stirring. Furthermore, the samples were precipitated with ethanol (preheated to 60°C) and the supernatant was filtrated through fritted crucibles containing Celite filter aid. The filtrates were collected for analysis of the hydrolyzable starch as glucose. The residue in the crucibles was washed with ethanol and dried overnight. Dried residue was ground in a mill and used for determination of RS after solubilization in 2M KOH (Siljeström et al 1988).

Statistical Analysis

All final data and correlation coefficients were calculated using Excel 97 on a PC.

RESULTS AND DISCUSSION

Using method 1, we analyzed test portions of nine types of Oatrim muffins for starch and TDF with or without DMSO treatment before enzyme incubation. Data show that the starch values for the DMSO-treated set were 64.03 to 73.73 g/100 g of dry weight, while the values for the untreated set were 54.90 to 71.75 g/100 g of dry weight, with an average standard deviation of ≤ 2 (Table II). The values for TDF for the DMSO-treated set were 2.74 to 6.51 g/100 g of dry weight, and for the untreated set were 3.62 to 16.17 g/100 g of dry weight. The amount of starch (from amylose and amylopectin) and dextrin (from Oatrim) in the recipe is 70.16 to 73.2 g/100 g of dry ingredients. These are very close to the analytical values obtained for glucose, expressed as starch for all muffins treated with DMSO, indicating the effectiveness of this reagent for solubilizing all starches. The β -glucan (from Oatrim) is the primary fiber source and its contribution to TDF content is 0.34, 1.87, and 3.39 g/100 g of dry ingredients for the low, moderate, and high subgroups, respectively. Resistant starch for any test muffin may be calculated according to method 1A using the starch values or method 1B using the TDF values from the two test sets (i.e., with or without DMSO treatment). As expected, the RS content increased proportionally with the level of amylose. To

evaluate the results on the RS data obtained from method 1, we also analyzed the same batch of muffins using methods 2 and 3. Method 3 employed simulated physiological conditions and has a high correlation with in vivo values from the literature for real foods (Åkerberg et al 1998), whereas methods 1 and 2 used strictly analytical parameters (e.g., higher temperatures and shorter reaction time). Method 2, however, is less labor intensive and more cost effective. A procedure similar to method 2 had been used to estimate RS content of selected cooked dried legumes at pretreatment temperatures of 121 and 130°C , the higher temperature being necessary for the type of RS found in legumes. A summary of the comparisons is given in Table III. For strictly analytical evaluation, values reported on a dry weight basis are more appropriate; however, for interpreting physiological effects from human studies, values reported on an as-eaten basis are more meaningful, especially when the moisture content of the muffins fell into three distinct subgroups. The low β -glucan had an average of 32%, the moderate 35%, and high subgroup 43%. The RS content for AP and a 1:1 mix of standard corn starch and high-amylose corn starch (MX) group are highest for method 3, but comparable for the AM group between methods 2 and 3. The squared correlation coefficients were determined for the three methods (Table IV). For all methods, regardless of the amount of β -glucan in the muffins, the AP group had the lowest, MX group had intermediate, and AM group had the highest RS content. Method 1 gave the lowest RS values for all three groups when compared with the other two methods. This may be due to the fact that, for this type of food, the initial heating with α -amylase at 100°C may have solubilized some of the RS; therefore, the difference between the treatments is less. Methods 2 and 3 had the best correlation for all muffins tested ($r^2 = 0.96$ dwb; 0.97 as-eaten). In fact, for the AM group alone the correlation is even better ($r^2 = 0.99$ as-eaten).

CONCLUSIONS

For estimating RS content of food, such as muffins prepared from starch with varying amounts of amylose and amylopectin, different methods gave somewhat different results. For method 1, the difference between TDF obtained from samples treated with and without DMSO afforded more consistent values for RS within any given group of muffins. Method 3 is the preferred method; however, it is also time-consuming and costly compared to the other two methods. For routine and high-volume analysis of food products based on flour or high-amylose starch, method 2 may be an adequate substitute for method 3.

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