Determination of Starches and Dietary Fiber Polysaccharides in Cooked Dried Beans: Comparison of Different Temperatures and Dimethyl Sulfoxide Treatments

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Cooked dried beans contain an appreciable amount of starches that escape digestion by human gastrointestinal enzymes. Such "resistant starch" possesses physiological characteristics similar to those of certain types of dietary fiber. Two brands of five dried beans (black beans, Great Northern beans, navy beans, pink beans, and pinto beans) were analyzed after cooking, using two different autoclave temperatures with or without dimethyl sulfoxide (DMSO). Starch as glucose and dietary fiber polysaccharides (DFP) as neutral sugars were determined according to a modified fiber method developed in this laboratory. Results showed that autoclaving at 130 °C (treatment I) and autoclaving at 121 °C in the presence of DMSO (treatment II) were quite similar and yielded higher starch and lower DFP values as compared to autoclaving at 121 °C (treatment III). The feasibility of estimating resistant starch in cooked dried beans by measuring the glucose present in enzyme digestates from two different starch solubilization temperatures was demonstrated.

Keywords: *Resistant starch; dietary fiber analysis; neutral sugars*

INTRODUCTION

It is now generally accepted that some foods contain starches that are digested at different rates and to varying extents by human gastrointestinal enzymes (Englyst and Kingman, 1990; McBurney, 1991; Silvester et al., 1995; Aman et al., 1995). The term "resistant starch" has been defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1992). Reports on the effects of resistant starch on fecal bulk and fermentation-dependent events or metabolic indexes (Phillips et al., 1995; Ranganathan et al., 1994) showed that fermentation characteristics of different forms of resistant starch are just as complex as those of nonstarch polysaccharides. With a physiological definition in place, analysts are again faced with the task of determining resistant starch in vitro, as has been the case with dietary fiber. Measurement of resistant starches is usually carried out as an integral part of dietary fiber or nonstarch polysaccharide determination. Variations of the method developed by Englyst and co-workers have appeared in the literature during the past decade (Englyst and Cummings, 1984; Berry, 1986; Muir and O'Dea, 1992; Saura-Calixto et al., 1993). For complete solubilization of starch either before or after hydrolytic enzyme incubation, test materials were without exception treated with either KOH or dimethyl sulfoxide (DMSO). Champ (1992) found that resistant starch yields in test samples were very dependent on the methods used in an interlaboratory study.

As part of the validation process for a simplified dietary fiber method developed in our laboratory, comparisons were made with two other methods (Lee et al., 1992; Al-Hasani et al., 1993) for the determination of total dietary fiber (TDF) in cooked dried legumes (Li, 1995). Results indicated that our method and that of Al-Hasani gave similar and much lower values for TDF when compared to AOAC Method 991.43. More extensive comparison and assessment were also made of the difference between five fiber methods (AOAC Methods 985.29, 991.43, and 992.16; Englyst's method; and the simplified method/treatment I) for TDF analysis of cooked dried legumes by Mongeau and Brassard (1994). They also found that our simplified method consistently gave lower TDF values than AOAC Methods 985.29 and 991.43, an indirect indication that more starches were removed with this method. In our continuing effort to investigate the extent of starch hydrolysis in dietary fiber determination and to estimate the relative amount of resistant starch in cooked dried legumes, we reanalyzed 10 freeze-dried samples from previous studies, this time for starches and dietary fiber polysaccharides (DFP) using two different autoclave temperatures with or without DMSO treatment prior to enzyme hydrolysis.

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MATERIALS AND METHODS

Samples and Sample Preparation. Dried beans of national brands were purchased locally in and around Beltsville, MD. Among them were black beans, Great Northern beans, navy beans, pink beans, and pinto beans. Beans were soaked and cooked according to package instructions. In general, 200 g of material was soaked in boiling water for 1 h and cooked until done (easily crushed between the fingers). Cooked beans were freeze-dried and ground in a Wiley mill to pass through a 30-mesh screen. Samples were further dried under vacuum at 60 °C and stored at room temperature.

Pretreatment before Enzyme Hydrolysis. Duplicate portions of cooked, ground, and dried bean (500 mg each) were weighed into 50-mL Teflon tubes and treated as follows: (1) suspended in 25 mL of deionized water and autoclaved at 130 °C for 1 h or (2) suspended in 2 mL of DMSO, heated in an oven at 100 °C for 30 s, mixed vigorously, and heated for an additional 30 min. Deionized water (25 mL) was added, and the sample was autoclaved at 121 °C for 1 h or (3) suspended in 25 mL of deionized water and autoclaved at 121 °C for 1 h.

Determination of Starches. After autoclaving, the samples were cooled to about 60 °C, 2 mL of enzyme solution [3 mg of

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Table 1. Starch (Total and Resistant) and DFP Contents (Grams per 100 g of Dry Weight) of Cooked Dried Beans[a]

	starch			resistant starch,	DFP		
sample	treatment I	treatment II	treatment III	treatments I−III	treatment I	treatment II	treatment III
black beans							
brand A	24.70	28.92	12.29	12.41	22.04	18.95	30.46
brand B	32.92	36.60	17.53	15.39	15.18	11.41	28.56
Great Northern beans							
brand A	33.38	31.30	22.53	10.85	17.19	18.16	29.09
brand B	30.80	28.85	18.44	12.36	15.98	19.07	26.90
navy beans							
brand A	25.25	26.67	14.49	10.75	24.59	19.65	34.20
brand B	33.60	31.51	13.59	20.01	14.13	16.28	31.11
pink beans							
brand A	37.72	30.59	20.19	17.53	13.40	16.33	26.61
brand C	34.61	33.50	17.92	16.69	13.04	17.18	27.14
pinto beans							
brand A	35.14	31.24	24.01	11.13	15.75	17.38	27.57
brand B	39.99	31.49	19.51	20.48	14.39	17.95	26.39

[a] Treatment I, autoclaved at 130 °C; treatment II, autoclaved at 121 °C in the presence of DMSO; treatment III, autoclaved at 121 °C. Values are means of duplicate determinations.

amyloglucosidase (Boehringer-Mannheim No. 208-469) in 1 mL of 4 M acetate buffer (pH 4.8) and 1 mL of deionized water] was added, and the tubes were placed in an incubator at 55 °C for 2 h with occasional mixing. Aliquots (0.1 mL) of supernatant from the enzyme digestates were removed, dried, and derivatized for glucose determination by gas−liquid chromatography (GLC). Starch content was calculated as the amount of glucose multiplied by 0.90. For detailed procedure refer to Li (1996).

Isolation of Dietary Fiber Residues. The mixtures remaining after the removal of small amounts of supernatant for starch analysis were diluted with 4 volumes of 95% ethanol to precipitate any polysaccharides and other materials that were solubilized during enzyme incubation. The final mixtures were filtered through sintered glass crucibles matted with Celite filtering aid. The residues were rinsed with dilute ethanol and acetone, dried in a 100 °C oven, cooled in a desiccator, and weighed.

Determination of DFP. One set of residues from each duplicate per treatment as isolated from dilute alcohol was hydrolyzed in 12 M H_2SO_4 at 35 °C for 1 h. Following dilution with water to give a final strength of 2 M, further hydrolysis of the polysaccharides took place during 2 h of heating at 100 °C. A second set of residues was hydrolyzed in only 2 M H_2SO_4 for 2 h. Neutral sugars in the resulting hydrolysates were derivatized to their alditol acetates and quantitated by GLC. DFP content was calculated as the sum of neutral sugars multiplied by 0.89. For detailed procedure refer to Li (1996).

Statistical Analysis. All final data and regression coefficients were calculated using Microsoft Excel version 4.0 on a Power Macintosh.

RESULTS AND DISCUSSION

Starch and DFP. In Table 1, total starch and DFP contents of two brands of five dried beans after cooking are given for three different treatments. For all samples tested, autoclaving at 130 °C (treatment I) prior to enzyme hydrolysis or autoclaving at 121 °C in the presence of DMSO (treatment II) gave higher starch and proportionally lower DFP values as compared to autoclaving at 121 °C (treatment III). This indicates that treatments I and II led to extensive if not complete solubilization of the starches in the test samples; however, treatment I is preferred because it gave highest starch and lowest DFP values for 7 of the 10 samples tested, and it does not require the use of DMSO as is often the case with all other methods for measuring resistant starch. In an earlier study, a simplified fiber method (treatment III) was modified by incorporating 130 °C autoclaving temperature (treatment I) as part of TDF determination in cooked dried legumes (Li, 1995) after it was shown that treatment III, as used in the original method, was inadequate for solubilizing all of the starches in legumes. More recent data showed that autoclaving at 121 or 130 °C in the presence of DMSO gave comparable results; therefore, treatment II with the lower temperature was chosen for the present study. Overall, starch values ranged from 24.7 to 40.0 g/100 g of dry weight for treatment I, from 26.7 to 36.6 g/100 g for treatment II, and from 12.3 to 24.0 g/100 g for treatment III.

Resistant Starch and Neutral Sugars. An estimation of resistant starch may be calculated by subtracting the amount of starch in treatment III from that in treatment I for each cooked dried bean. The resulting values for resistant starch content varied between 10.8 g/100 g for Great Northern beans and navy beans of brand A and 20.5 g/100 g for pinto beans of brand B.

Differences between brands were greater than that between types in the case of navy beans and pinto beans. DFP as the sum of neutral sugars ranged from 13.0 to 24.6 g/100 g for treatment I, from 11.4 to 19.6 g/100 g for treatment II, and from 26.4 to 34.2 g/100 g for treatment III. Individual neutral sugars in the dietary fiber residues are given in Table 2. All beans contain very little rhamnose (<0.2 g/100 g), small amounts (0.2−0.8 g/100 g) of fucose and mannose, slightly more (0.8−2.7 g/100 g) galactose and xylose, and even more arabinose (3.8−6.3 g/ 100 g). The amount of glucose varied greatly (1.0−27.9 g/100 g) depending on the pretreatment and the acid hydrolysis conditions used. Concentrated H_2SO_4 (12 M) is generally used to hydrolyze cellulose in dietary fiber residues, while hydrolysis in dilute H_2SO_4 (2 M) affords only glucose from noncellulosic polysaccharides, including starch remaining in the fiber residues. Difference between glucose values obtained from these two hydrolysis conditions is supposed to give an estimation of the cellulose content and the amount of resistant starch in the fiber residue under different treatments of a sample; however, it varied not only between treatments but also between brands of beans (data not shown). The differences were much greater than those calculated from the starch values. By determining the amount of glucose present in the enzyme digestate and in the fiber residues under various conditions, we were able to distinguish the amount of starch that was hydrolyzed enzymatically from that which was isolated as part of

Table 2. Neutral Sugars Content (Grams per 100 g of Dry Weight) of DFP of Cooked Dried Beans[a]

sample	treatment	fucose	mannose	galactose	xylose	arabinose	glucose
black beans							
brand A	I	0.22	0.63	0.89	1.99	4.40	16.64
	II	0.24	0.67	1.00	2.10	5.40	11.88
	III	0.22	0.43	0.85	1.89	4.97	25.88
brand B	I	0.29	0.29	0.89	1.96	4.77	8.86
	II	0.28	0.44	0.89	1.80	5.06	4.35
	III	0.27	0.76	1.00	1.31	5.20	23.28
Great Northern beans							
brand A	I	0.32	0.67	1.21	2.71	5.59	8.81
	II	0.35	0.56	0.87	1.55	4.89	12.02
	III	0.31	0.75	1.14	2.34	5.91	22.23
brand B	I	0.32	0.61	1.00	2.29	4.97	8.76
	II	0.32	0.86	1.04	2.02	5.42	11.60
	III	0.31	0.48	0.93	2.09	5.26	21.16
navy beans							
brand A	I	0.45	0.58	1.12	2.55	6.16	16.78
	II	0.35	0.63	0.95	1.76	4.81	13.42
	III	0.45	0.41	1.08	2.12	6.44	27.92
brand B	I	0.41	0.63	0.95	2.20	4.89	6.80
	II	0.40	0.79	0.98	2.19	5.30	8.51
	III	0.41	0.70	0.99	2.03	5.22	25.61
pink beans							
brand A	I	0.29	0.52	0.92	1.91	4.48	6.92
	II	0.32	0.60	0.91	1.76	5.03	9.58
	III	0.29	0.53	0.91	1.97	4.97	21.23
brand C	I	0.27	0.35	0.76	1.56	3.84	7.88
	II	0.35	0.65	0.96	1.91	5.07	10.22
	III	0.30	0.46	0.97	1.92	5.40	21.43
pinto beans							
brand A	I	0.22	0.48	0.88	2.22	4.52	9.38
	II	0.26	0.57	0.90	1.86	5.14	10.80
	III	0.31	0.77	1.08	1.77	5.81	21.06
brand B	I	0.29	0.41	0.87	1.69	4.56	8.28
	II	0.36	0.50	0.88	1.67	4.84	11.76
	III	0.30	0.28	0.84	1.74	5.04	21.45

[a] Treatment I, autoclaved at 130 °C; treatment II, autoclaved at 121 °C in the presence of DMSO; treatment III, autoclaved at 121 °C.

dietary fiber; the latter was more difficult to quantify, as mentioned above.

Statistical Evaluation. Each bean sample was analyzed in duplicate with an average coefficient of variation <2.5% for starch and <1% for DFP (sum of neutral sugars). The resistant starch values correlate negatively with DFP with $r = -0.73$ for treatment I and $r = -0.58$ for treatment II. This indicates that treatment I minus treatment III gives a better estimate of the resistant starch than treatment II minus treatment III.

Conclusion. Significant amounts of resistant starch, almost half of the total starches, were found in cooked dried beans, and the resistant starches are likely to be the physically inaccessible and retrograded starches as described by Englyst and Kingman (1990). Even though resistant starches pass through the small intestine and are fermented in the colon similarly to other nonstarch polysaccharides, their pattern of fermentation (Englyst et al., 1987) and their effect on colonic environment are different from NSP (Phillips et al., 1995). In light of these observations, resistant starches should be separated and determined independently during dietary fiber analysis and not be left as part of dietary fiber. In this study, we demonstrated the feasibility of estimating resistant starch in cooked dried beans by measuring the glucose present in enzyme digestate from two different starch solubilization temperatures. We also showed that cooked dried beans are good sources for both resistant starch and DFP, which have been shown to possess healthful benefits to consumers. Legumes in general have the lowest glycemic index as compared to cereal grains and other starchy foods (Wolver, 1990).

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