Adaptation of the AOAC 2011.25 Integrated Total Dietary Fiber Assay To Determine the Dietary Fiber and Oligosaccharide Content of Dry Edible Beans

Adrienne E. Kleintop,[†] Dimas Echeverria,[†] Leslie A. Brick,[†] Henry J. Thompson,[‡] and Mark A. Brick*[,†]

[†]Department of Soil and Crop Sciences and [‡]Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, Colorado 80523, United States

ABSTRACT: Dietary fiber (DF) has important health benefits in the human diet. Developing dry edible bean (*Phaseolus vulgaris* L.) cultivars with improved DF and reduced nondigestible oligosaccharide content is an important goal for dry bean breeders to increase consumer acceptance. To determine if genetic variation exists among dry bean cultivars for DF, two populations of diverse dry bean cultivars/lines that represent two centers of dry bean domestication were evaluated for dietary fiber using the Integrated Total Dietary Fiber Assay (AOAC 2011.25). This assay was adapted to measure water insoluble dietary fiber, water soluble dietary fiber, oligosaccharides raffinose and stachyose, and the calculated total dietary fiber (TDF) content of cooked dry bean seed. The AOAC 2011.25 protocol was modified by using a quick, simple, and sensitive high-performance liquid chromatography method paired with an electrochemical detection method to separate and quantify specific oligosaccharides, and using duplicate samples as replicates to generate statistical information. The TDF of dry bean entries ranged from 20.0 to 27.0% in population I and from 20.6 to 25.7% in population II. Total oligosaccharides ranged from 2.56 to 4.65% in population I and from 2.36 to 3.84% in population II. The results suggest that significant genetic variation exists among dry bean cultivars/lines to allow for genetic selection for improved DF content in dry beans and that the modifications to the AOAC 2011.25 method were suitable for estimating DF in cooked dry edible beans.

KEYWORDS: *dry beans, Phaseolus vulgaris L., total dietary fiber, insoluble dietary fiber, soluble dietary fiber, oligosaccharides, raffinose, stachyose, HPAE-PAD, integrated total dietary fiber assay*

■ INTRODUCTION

Dietary fiber in the human diet has been shown to have important health benefits and implications for chronic disease prevention. A recent review concluded that DF intake was associated with reduced coronary heart disease, stroke, hypertension, diabetes, obesity, and gastrointestinal disorders.[1] Fiber intake has been shown to improve insulin sensitivity in both diabetics and nondiabetic individuals, lower blood pressure and blood cholesterol levels, and facilitate weight loss in obese patients.[1−6] Park et al. reported that DF intake was associated with reduced cardiovascular- and infectious respiratory-related deaths in a large prospective study.[7] A European study reported an inverse relationship between DF intake and colorectal cancer, suggesting that doubling dietary fiber intake in the lowest intake category could result in a 40% reduction in colorectal cancer risk.[8] The scientific evidence clearly demonstrates that DF is beneficial to human health.

Despite the important health benefits of DF in the prevention of chronic diseases, the level of intake of DF in the United States is well below adequate intake levels of 25−38 g day^{-1} person^{-1}.[9] Dry edible beans have been recommended as a means of increasing the level of DF in the human diet.[10,11] In a comparison of 70 different food items, legumes, including dry edible beans, were found to have the highest DF content.[12] In addition to being high in fiber, dry edible beans are also high in protein, supply essential vitamins and minerals, and are very low in fat and cholesterol.[10,11] Mitchell et al. reported that consumption of $^1/_2$ cup of dry edible beans or peas (*Pisum sativum* L.) per day (26 g dry weight equivalent) increased the dietary intake of protein,

folate, iron, zinc, and magnesium and that consumers of dry edible beans or peas had higher fiber intake than nonconsumers.[11] However, dry beans also contain undesirable α-galactosides, also known as the raffinose family of oligosaccharides, which include raffinose, stachyose, and verbascose.[13−15] The oligosaccharide fraction is a concern in the human diet because they are fermented in the intestinal tract and result in flatulence and digestive discomfort.[13−15] Because of the negative aspects associated with oligosaccharides, decreasing their content is of interest in enhancing the acceptability of beans in the human diet. Plant breeders are interested in reducing the oligosaccharide fraction and increasing the benefits of DF in dry beans. Consequently, methods for evaluating genetic diversity and making selections in segregating breeding populations will require accurate and efficient analytical methods for quantifying their content.

Definitions and analytical methods for characterizing and measuring components of DF in food have evolved over the past 60 years. A precise definition of DF has been difficult because it contains many plant components with diverse chemical compositions.[16−20] Hipsley is credited with the first use of the term DF in 1953.[21] In the mid-1970s, Trowell defined the complex and variable mixture of dietary fiber as "plant polysaccharides and lignin which are resistant to hydrolysis by

the digestive enzymes of man".[22] This definition served as the foundation of subsequent related definitions and analytical methods for decades. In the early 1990s, a consensus emerged that nondigestible oligosaccharides and resistant starch function as dietary fiber and should be included in the definition of DF, prompting attempts to expand the definition.[18,19,23] During this time, the authority responsible for determining global food standards and guidelines, the Codex Alimentarius Commission, undertook the task of redefining DF because the guidelines have implications for food labeling, reference nutrient levels, and health claims.[16,24−27] This task concluded with the adoption of a new definition of DF in 2009 by the Codex Committee on Nutrition and Foods for Special Dietary Uses.[18] The definition states that "Dietary fiber consists of carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans" and classified into three categories of carbohydrate polymers with demonstrated physiological health beneficial properties, including (1) consumed as naturally occurring in the food, (2) obtained from raw food by physical, enzymatic, or chemical means, and (3) synthetically produced.[17,18,28] This expanded definition resulted in a subsequent review of available analytical methods and whether the existing methods could define the fiber components included in the new definition.[23,28] An integrated total DF analytical method was developed by McCleary et al. to comply with the 2009 CODEX definition and accurately measure total dietary fiber (TDF) as calculated by insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and nondigestible oligosaccharide content.[16−18,28] This integrated method was subjected to interlaboratory evaluations and initially accepted as AOAC 2009.01.[17,18,28] Additional interlaboratory evaluations for TDF, IDF, and SDF resulted in the acceptance of AOAC method 2011.25.[29]

Plant breeders are interested in improving the health benefits of beans by increasing the most beneficial DF and reducing the oligosaccharide fraction. Our objective was to adapt the AOAC 2011.25 method to dry edible bean seed and evaluate beans in a form in which they are commonly consumed. The long-term goal of our research is to use these methods in a breeding program to develop dry bean cultivars with improved health benefits and reduced digestive problems to increase the rate of consumption. The specific objectives of this research were (1) to adapt the AOAC 2011.25 method to assess TDF in cooked dry edible bean seed and (2) to report the DF and oligosaccharide contents of two sets of genetically diverse dry edible bean cultivars/lines using the AOAC 2011.25 method.

■ MATERIALS AND METHODS

AOAC 2011.25 Integrated Total Dietary Fiber Assay. The total integrated dietary fiber content was quantified in dry edible bean lines according to the AOAC 2011.25 method using a commercial assay kit (K-INTDF) purchased from Megazyme International (Wicklow, Ireland). The assay was conducted with modifications as noted in this report following the manufacturer's instructions for the 2011.25 Codex method, which included enzymatic digestion with an α-amylase/AMG mixture, two gravimetric filtrations, and high-performance liquid chromatography (HPLC) analysis to quantify oligosaccharide content.[29] A sample preparation protocol was developed to simulate commercial processed canned beans that are commonly consumed in the United States.

Plant Materials. Seeds from two diverse populations of dry bean cultivars/lines (entries) were evaluated in this study. The first population (population I) consisted of entries from 31 different commercial varieties obtained from the U.S. Department of Agriculture

Common Bean Coordinated Agricultural Project (BeanCAP).[30] This population consisted mainly of bean cultivars commonly grown in the United States. The seed for this population was grown in a single greenhouse environment in 2010 by J. D. Kelly (Department of Crop Science, Michigan State University, East Lansing, MI). The second population (population II) was used to validate if the range of values observed in the first population were similar to the second. Population II included entries from 25 diverse dry bean cultivars/lines commonly used as parents in genetic studies of dry bean. Seeds from this population were grown in the greenhouse by scientists that developed or utilized the lines in genetic studies. Seeds from population II entries were grown in diverse greenhouse environments by the original scientists that provided the seed. All entries from both populations were chosen to represent germplasm in different market classes (differing in seed size and color), and genetic races of beans as classified by Singh that represent the two world Centers of Domestication for common bean, namely, Middle American and Andean.[31]

Sample Preparation. Two replicates of approximately 1 g of whole dry bean seed samples per entry were weighed and dried for 24 h in a convection oven at 40 °C to bring all seed to a moisture content of approximately 6%. After drying, seed samples where weighed again and transferred to 50 mL conical tubes. Eight milliliters of Milli-Q water was added to the conical tubes, and seeds were soaked for 14 h at room temperature. The tubes were then placed in an autoclave where they were autoclaved for 65 min at 115 °C with a pressure of 76 kPa to simulate the cooking process of a canned bean product. The contents of the tubes were fully homogenized using a Polytron PT10/35 with an S-type probe, for approximately 15 s on speed 6. The probe was washed twice with 6 mL of Milli-Q water in a VWR Culture tube 17 mm × 100 mm after homogenization to remove all seed particles. The liquids from the two washings were transferred to the original 50 mL conical tube containing the homogenized seed sample. The final sample volume was approximately 20 mL. The conical tubes with the homogenized seed samples were stored at −80 °C until they were analyzed.

Enzymatic Digestion with Pancreatic α-Amylase/AMG Mixture. Prior to enzymatic digestion, homogenized seed samples in conical tubes were thawed at room temperature and transferred to a 100 mL glass bottle. A 20 mL volume of maleate buffer (pH 6.0) with an α-amylase/AMG mixture was added to each glass bottle containing the homogenized seed samples to bring the total volume to 40 mL. Two blank glass bottles without seed samples that contained just 20 mL of Milli-Q water and 20 mL of the enzymatic mix were also run with each assay to measure any contribution from reagents to residues. The glass bottles with contents were placed in a Grant OLS 200 shaking incubation water bath and incubated for 16 h at 37 °C at 150 rpm in an orbital motion. After 16 h, the glass bottles were removed and the pH was adjusted to approximately 8.0 with the addition of 3.0 mL of 0.75 M Trizma base solution. The bottle caps were loosened slightly, and samples were then incubated in a water bath at 95−100 °C for 20 min. The samples were removed and allowed to cool to 60 °C, and 0.1 mL of a protease solution was added to each bottle. The glass bottles were placed into a shaking incubation water bath and incubated at 60 °C for 30 min. Following incubation, the pH of each sample was adjusted to approximately 4.3 via the addition of 4.0 mL of 2 M acetic acid and returned to the 60 °C water bath for immediate gravimetric filtration.

First Gravimetric Filtration (IDF Determination). Fritted glass crucibles (Pyrex, 50 mL, coarse pore size, ASTM 40−60 μm) were prepared by adding approximately 1.0000 g ± 5 mg of Celite to each crucible and dried at 130 °C overnight. The crucibles were removed and placed in a desiccator (airtight with SiO$_2$ desiccant) for 1 h, and then the mass of the crucible containing Celite was recorded to the nearest 0.1 mg. The precipitate in the 100 mL glass bottles from the enzymatic digestion contained the insoluble dietary fiber (IDF) portion of the sample. To collect the precipitate, the crucibles were wetted with 15 mL of 78% (v/v) EtOH under vacuum suction to distribute the Celite at the base of the crucible. The contents of the glass bottles were vacuum-filtered through the crucible into a 1 L flask, and the bottles were washed twice with 10 mL portions of 60 °C Milli-Q water to retain all sample particles being transferred to the crucible. The filtrate and washings (~80 mL) were collected for the second gravimetric filtration (SDF

Determination). The IDF residue in the crucible was then washed sequentially with 15 mL portions of 78% (v/v) EtOH and 95% (v/v) EtOH, and acetone, and the washings were discarded. The crucibles containing the IDF residue were dried in a convection oven at 105 °C overnight. The crucibles were removed, placed in a desiccator for 1 h, and then weighed to the nearest 0.1 mg. To determine the mass of the residue, the mass of the crucible and Celite was subtracted from this value. Residues were saved for ash and protein corrections.

Second Gravimetric Filtration (SDF Determination). The filtrate from the IDF filtration was preheated to 60 °C, and then 320 mL of 60 °C 95% (v/v) EtOH was added, mixed, and transferred quantitatively to a 500 mL Pyrex glass bottle. The SDF was allowed to precipitate for 60 min at room temperature. The contents of the 500 mL bottle were poured into a prewetted fritted crucible (containing Celite) under vacuum suction as described in First Gravimetric Filtration (IDF Determination). The SDF was retained on the crucible as filtrate with the oligosaccharides passed through the filter and collected into a 1 L flask. The filtrate volume was recorded quantitatively, and 45 mL of the filtrate was transferred to a 50 mL conical tube and stored at −80 °C for later analysis of oligosaccharide content. The residue was washed sequentially with two 15 mL portions of 78% (v/v) EtOH, 95% (v/v) EtOH, and acetone, and the washings were discarded. The crucibles containing the SDF residue were dried in a convection oven at 105 °C overnight. The crucibles were removed, placed in a desiccator for 1 h, and then weighed to the nearest 0.1 mg. To determine the mass of the residue, the mass of the crucible and Celite was subtracted from this value. Residues were saved for ash and protein corrections.

IDF and SDF Residue Analysis for Ash and Protein Corrections. The IDF and SDF residues were analyzed for ash and protein to make weight corrections for the calculation of fiber values. Replicate 1 was used to calculate ash, and replicate 2 was used to calculate protein. The ash content of the residues was determined by incineration of the residue at 495 °C for 5 h. The crucibles were removed, placed in a desiccator for 1 h, and then weighed to the nearest 0.1 mg. To determine the mass of the ashes, the mass of the crucible and Celite was subtracted from this value and corrected with the blank ashes. For protein analysis, the residue and Celite were quantitatively transferred from the crucible to a glass vial and homogenized with a glass rod. The glass vial with contents was stored in a desiccator until they were analyzed. The nitrogen content of the homogenized residues was analyzed by the Dumas method using LECO TruSpec equipment, and the final nitrogen content of the residue was corrected for the weight of Celite in the homogenate. A conversion factor of 6.25 was used to convert nitrogen content to protein content.

Calculations for Insoluble and Soluble Dietary Fiber Content. The percent IDF or SDF was calculated with the equations %IDF = $(R_{IDF} - A_{IDF} - P_{IDF} - B_{IDF})/S \times 100$, where R_{IDF} is the residue dry weight, A_{IDF} is the ash dry weight in the residue, P_{IDF} is the protein dry weight in the residue, B_{IDF} is the residue blank, and S is the seed sample dry weight, and %SDF = $(R_{SDF} - A_{SDF} - P_{SDF} - B_{SDF})/S \times 100$, where R_{SDF} is the residue dry weight, A_{SDF} is the ash dry weight in the residue, P_{SDF} is the protein dry weight in the residue, B_{SDF} is the residue blank, and S is the seed sample dry weight.

Analysis of Raffinose and Stachyose Content. The filtrate retained after the second gravimetric filtration contained the nondigestible oligosaccharides. Oligosaccharides in the filtrate were analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The analysis was performed with a Shimadzu instrument (Shimadzu Corp., Kyoto, Japan) equipped with an SCL-10Avp system controller, an LC10ATvp pump, a DGU-20A5 online degasser, and an SIL-10A autosampler with a 20 μL fixed loop. The oligosaccharides were separated on a Dionex CarboPac PA10 anion-exchange resin analytical column (4 mm × 250 mm) with a Dionex CarboPac PA10 guard column (4 mm × 50 mm) (Dionex, Sunnyvale, CA). The mobile phase consisted of a 140 mM NaOH solution at a flow rate of 1.0 mL/min under isocratic conditions. The mobile phase was prepared by diluting a carbonate-free 50% (w/w) NaOH solution in Milli-Q water. The detection was accomplished by an ED40 electrochemical detector with a gold working electrode and a Ag/AgCl reference electrode (Dionex). Pulsed amperometric detection was

conducted with the following pulse settings, E_1 = 100 mV (t_1 = 400 ms), E_2 = −2000 mV (t_2 = 20 ms), E_3 = 600 mV (t_3 = 10 ms), and E_4 = −100 mV (t_4 = 60 ms). The data acquisition time for each sample was 15 min. Commercial raffinose and stachyose were purchased from Sigma-Aldrich (St. Louis, MO) and were used as external standards to identify and quantify each saccharide based on its retention time and peak height. The correlation coefficient (r) of the detector response versus standard concentrations was greater than 0.99 for all standards. Chromatographic data were collected, plotted, and analyzed using Class-VP version 7.2.1.

Statistical Analysis. An analysis of variance was conducted to compare entry means on all variables using the Proc GLM procedure in SAS version 9.2 (SAS Institute Inc., Cary, NC). Two replicates per entry were used for all variables except as noted. Tukey's multiple-mean comparison method ($p < 0.05$) was used to determine significance among entry means for all variables. To determine significance between overall entry means between Middle American and Andean Centers of Domestication (COD), an analysis of variance was conducted with entry codes for COD, and a single degree of freedom F-test was conducted to compare the means for each COD. Information obtained from these F-tests must be carefully interpreted because there were unbalanced sets of entries with 6 Andean versus 25 Middle American entries evaluated in population I and 4 Andean versus 21 Middle American entries compared in population II.

■ RESULTS AND DISCUSSION

Modifications to AOAC 2011.25. To the best of our knowledge, this report represents the first publication on the use of the newly developed integrated total dietary fiber assay AOAC 2011.25 to evaluate diversity in cultivars of dry edible bean seed. The 2009.01 method has also previously been reported for the analysis of wheat (*Triticum* sp.), rye (*Secale cereal* L.), and various bread and bakery products.[32−34] Several modifications to AOAC method 2011.25 were introduced in our analysis to facilitate the evaluation of a high sample number and to accommodate dry bean seed as it would be prepared as a canned product. The analysis of DF using the modified AOAC 2011.25 method for each cooked sample took approximately 2 days (one run) to complete for IDF, SDF, stachyose, and raffinose, assuming two people worked full time (8 h/day). We were able to analyze 14 samples per run, where each sample is considered an experimental unit (one replicate of one entry). Higher sample quantity could be achieved with more laboratory equipment and space.

The AOAC 2011.25 protocol uses duplicate samples (∼1 g each) to calculate DF in a food product. One duplicate sample is used to determine the ash content of the IDF and SDF residues, and the other duplicate sample is used to estimate the protein content of the residues. The ash and protein contents of the sample residues are then used in the final calculation for the IDF and SDF of the food product.[17] We also used two samples from each dry bean entry to estimate the DF. However, we treated each sample as a replicate and estimated ash in one replicate and protein in the alternate replicate. We then used the proportion of ash or protein content in the replicate in which it was measured to calculate its content in the alternate replicate after adjusting for the alternative sample dry weight. This resulted in two calculations for IDF and SDF, one from each sample that we treated as replicates for statistical purposes. This decision was made to streamline the AOAC 2011.25 protocol to reduce the amount of time and costly materials required to run large numbers of samples as would be required in a breeding program. Using this method, coefficients of variation (CVs) among all variables ranged between 2.9 and 14.8% for populations I and II (Tables 1). To compare the precision of using duplicate samples

Table 1. Coefficients of Variation among Three Experimental Sets of Data for Insoluble Dietary Fiber (IDF), Soluble Dietary Fiber (SDF), IDF+SDF, Stachyose, Raffinose, Total Oligosaccharides, and Total Dietary Fiber (TDF)

	CV (%)						
	IDF	SDF	IDF+SDF	stachyose	raffinose	oligos	TDF
check cultivars	3.6	6.8	1.8	5.97	8.18	5.61	1.5
population I	3.7	6.0	2.9	–	–	–	–
population II	5.4	7.4	4.4	6.38	14.76	6.23	4.3

Table 2. Insoluble Dietary Fiber (IDF), Soluble Dietary Fiber (SDF), IDF+SDF, Stachyose, Raffinose, Total Oligosaccharides, and Total Dietary Fiber (TDF) Content for Population I Dry Bean Entries[a]

entry	COD	seed type	IDF (%)[b]	SDF (%)[b]	IDF+SDF (%)[b]	stachyose (%)[c]	raffinose (%)[c]	oligos (%)[c]	TDF (%)[c]
A801	MA	carioca	16.2 A	6.1 CDEFGH	22.3 ABC	3.98	0.67	4.65	27.0
AC Early Red	MA	SR	14.5 ABCDEF	6.7 CDEFG	21.2 ABCDE	3.27	0.27	3.54	24.7
Avalanche	MA	navy	13.0 FGH	6.1 CDEFGH	19.1 EFG	3.20	0.57	3.77	22.9
Beryl	MA	GN	13.7 BCDEFG	6.9 CDEFG	20.5 BCDEF	2.62	0.47	3.09	23.6
Big Bend	MA	SR	14.6 ABCDEF	8.6 B	23.2 A	3.36	0.36	3.72	26.9
Black Knight	MA	black	14.5 ABCDEF	7.0 BCDEF	21.5 ABCDE	2.97	0.53	3.50	25.0
Blackjack	MA	black	14.5 ABCDEF	6.0 CDEFGH	20.5 BCDEF	3.19	0.57	3.76	24.3
Burke	MA	pinto	14.3 ABCDEFG	5.4 FGHI	19.8 DEF	3.26	0.73	3.99	23.8
Buster	MA	pinto	13.0 FGH	7.5 BCD	20.5 BCDEF	2.64	0.31	2.95	23.5
CDC Rosalee	MA	pink	13.5 CDEFGH	7.5 BC	21.0 ABCDEF	3.31	0.36	3.67	24.7
CRAN-09	Andean	cranberry	13.5 CDEFGH	6.9 CDEFG	20.4 BCDEF	2.33	0.32	2.65	23.1
Crestwood	MA	navy	13.0 FGH	6.7 CDEFG	19.7 DEF	2.74	0.49	3.23	22.9
Eclipse	MA	black	13.3 EFGH	7.1 BCDE	20.4 BCDEF	3.14	0.55	3.69	24.1
HY4181	MA	GN	13.4 DEFGH	3.4 JK	16.8 G	2.69	0.47	3.16	20.0
Ind. Jamaican Red	Andean	cranberry	15.6 ABC	4.4 JKI	20.0 CDEF	3.78	0.31	4.09	24.1
Lite Kid	Andean	LRK	14.5 ABCDEF	6.7 CDEFG	21.2 ABCDE	2.91	0.34	3.25	24.5
Marquis	MA	GN	13.5 CDEFGH	7.0 BCDEF	20.5 BCDEF	2.53	0.41	2.94	23.4
Merlot	MA	SR	15.7 AB	6.7 CDEFG	22.4 AB	2.29	0.27	2.56	25.0
MI Imp. Cranberry	Andean	cranberry	11.4 H	10.6 A	22.0 ABCD	2.32	0.35	2.67	24.7
Midland	MA	navy	15.3 ABCDE	3.4 K	18.6 FG	3.05	0.59	3.64	22.2
Midnight	MA	black	15.5 ABCD	4.9 HIJ	20.5 BCDEF	3.37	0.59	3.96	24.5
NW410	MA	pinto	14.1 ABCDEFG	6.1 CDEFGH	20.2 BCDEF	3.23	0.23	3.46	23.7
Othello	MA	pinto	14.4 ABCDEF	5.7 EFGHI	20.1 BCDEF	3.85	0.24	4.09	24.2
PK 915	MA	pink	15.0 ABCDEF	6.3 CDEFGH	21.3 ABCDE	2.70	0.40	3.10	24.4
Pompadour B	Andean	SB	14.0 BCDEFG	6.9 CDEFG	20.9 ABCDEF	2.80	0.38	3.18	24.1
Seabiskit	MA	navy	13.3 EFGH	5.9 DEFGHI	19.2 EFG	3.03	0.49	3.52	22.7
Starlight	MA	GN	15.1 ABCDEF	5.4 GHI	20.5 BCDEF	2.94	0.49	3.43	23.9
UCD 9623	MA	FdM	12.2 GH	7.4 BCD	19.6 DEF	2.79	0.33	3.12	22.7
UCD 9634	MA	pink	13.6 BCDEFG	6.4 CDEFGH	20.0 BCDEF	3.44	0.41	3.85	23.9
UI 537	MA	pink	14.5 ABCDEF	6.4 CDEFGH	20.9 ABCDEF	2.71	0.51	3.22	24.1
USWK-6	Andean	WK	14.7 ABCDEF	6.4 CDEFGH	21.1 ABCDEF	2.72	0.33	3.05	24.2
overall									
range			11.4–16.2	3.4–10.6	16.8–23.2	2.29–3.98	0.23–0.73	2.56–4.65	20.0–27.0
mean			14.1	6.4	20.5	3.01	0.43	3.44	24.0
CV			3.7	6.0	2.9	--	--	--	--
p value			<0.0001	<0.0001	<0.0001	--	--	--	--
center of domestication									
Andean			13.9	7.0	20.9	2.81	0.34	3.15	24.1
MA			14.2	6.3	20.4	3.05	0.45	3.51	23.9
p value			0.5392	0.1039	0.2245	--	--	--	--

[a]Abbreviations: COD, Center of Domestication; MA, Middle American; Andean, Andean South America; GN, great northern; WK, white kidney; LRK, light red kidney; SR, small red; FdM, Flor de Mayo; SB, sugar bean; Ind. Jamaican Red, Indeterminate Jamaican Red; MI Imp. Cranberry, Michigan Improved Cranberry. [b]Mean separations were performed using Tukey's multiple-comparison tests. Means with the same letter are not significantly different at $p < 0.05$. [c]Statistics for stachyose, raffinose, total oligosaccharides, and TDF content are not available because only one biological replicate was analyzed.

for each replicate with single samples for each replicate as described above, we also analyzed four different cultivars for DF

using duplicate samples for each replicate to compare coefficients of variation with those produced from populations I and II.

Table 3. Insoluble Dietary Fiber (IDF), Soluble Dietary Fiber (SDF), IDF+SDF, Stachyose, Raffinose, Total Oligosaccharides, and Total Dietary Fiber (TDF) for Population II Dry Bean Entries[a]

entry	COD	seed type	IDF (%)	SDF (%)	IDF+SDF (%)	stachyose (%)	raffinose (%)	oligos (%)	TDF (%)
115M	MA	black	14.9 A	3.3 G	18.2 BC	2.95 ABCDE	0.38 CDEFGH	3.33 ABC	21.6 B
AN37	MA	pinto	13.7 A	5.9 ABCDEF	19.6 ABC	2.92 ABCDE	0.39 CDEFGH	3.32 ABCD	22.9 AB
B98311	MA	black	13.7 A	5.9 BCDEF	19.6 ABC	2.69 ABCDE	0.38 CDEFGH	3.07 ABCDEF	22.7 AB
Bat93	MA	cream	12.4 A	5.6 CDEF	18.0 BC	2.95 ABCDE	0.37 CDEFGH	3.32 ABCD	21.3 B
Buster	MA	pinto	13.8 A	6.3 ABCDE	20.0 ABC	2.40 CDE	0.20 HIJK	2.60 CDEF	22.6 AB
CDRK	Andean	DRK	13.5 A	7.3 ABC	20.8 ABC	2.24 E	0.20 GHIJK	2.44 EF	23.2 AB
EMP507	MA	carioca	13.9 A	3.4 G	17.3 C	2.82 ABCDE	0.47 BCD	3.29 ABCD	20.6 B
G08263	MA	GN	13.7 A	5.4 DEF	19.1 ABC	2.98 ABCD	0.47 BCDE	3.44 AB	22.5 AB
Jaguar	MA	black	13.4 A	5.9 ABCDEF	19.3 ABC	2.91 ABCDE	0.41 CDEF	3.32 ABC	22.6 AB
Jalo-EEP558	Andean	yellow	12.4 A	7.5 AB	19.9 ABC	2.32 DE	0.04 K	2.36 F	22.3 AB
Lassen	Andean	WK	14.8 A	7.7 A	22.5 A	3.09 ABC	0.11 JK	3.20 ABCDE	25.7 A
Matterhorn	MA	GN	14.3 A	5.3 DEF	19.6 ABC	2.43 CDE	0.53 ABC	2.97 BCDEF	22.6 AB
P02630	MA	pinto	13.9 A	5.7 BCDEF	19.6 ABC	3.17 AB	0.67 AB	3.84 A	23.5 AB
P02647	MA	pinto	14.6 A	5.1 EFG	19.7 ABC	2.88 ABCDE	0.30 DEFGHIJ	3.18 ABCDE	22.9 AB
P07863	MA	pinto	14.0 A	6.3 ABCDE	20.3 ABC	2.71 ABCDE	0.35 CDEFGH	3.06 ABCDEF	23.4 AB
Puebla152	MA	black	14.1 A	6.1 ABCDEF	20.2 ABC	3.33 A	0.15 IJK	3.48 AB	23.7 AB
Red Hawk	Andean	DRK	12.9 A	7.0 ABCD	19.9 ABC	3.07 ABC	0.28 DEFGHIJ	3.35 ABC	23.2 AB
Roza	MA	pink	12.6 A	7.4 AB	20.0 ABC	2.47 BCDE	0.33 DEFGHI	2.79 BCDEF	22.8 AB
Sanilac	MA	navy	12.9 A	5.9 BCDEF	18.8 BC	2.77 ABCDE	0.40 CDEFGH	3.16 ABCDE	22.0 AB
Seahawk	MA	navy	13.9 A	5.0 EFG	18.9 BC	2.62 ABCDE	0.24 FGHIJ	2.86 BCDEF	21.7 B
SER22	MA	SR	13.8 A	4.3 FG	18.2 BC	2.96 ABCDE	0.19 HIJK	3.15 ABCDEF	21.3 B
Stampede	MA	pinto	14.8 A	6.4 ABCDE	21.2 AB	2.65 ABCDE	0.40 CDEFG	3.05 ABCDEF	24.3 AB
TLP19	MA	black	14.5 A	6.0 ABCDEF	20.5 ABC	2.84 ABCDE	0.69 A	3.53 AB	24.1 AB
Yolano	MA	pink	12.7 A	6.2 ABCDE	18.9 BC	2.26 DE	0.27 EFGHIJ	2.52 DEF	21.4 B
Zorro	MA	black	13.2 A	6.6 ABCDE	19.8 ABC	2.94 ABCDE	0.30 DEFGHIJ	3.25 ABCD	23.0 AB
overall[b]									
range			12.4–14.9	3.3–7.7	17.3–22.5	2.24–3.33	0.04–0.69	2.36–3.84	20.6–25.7
mean			13.7	5.9	19.6	2.77	0.34	3.11	22.7
CV			5.4	7.4	4.4	6.38	14.76	6.23	4.3
p value			0.0464	<0.0001	0.0027	<0.0001	<0.0001	<0.0001	0.0111
center of domestication[b]									
Andean			13.4	7.4	20.8	2.68	0.16	2.84	23.6
MA			13.8	5.6	19.4	2.79	0.38	3.17	22.6
p value			0.3275	<0.0001	0.0028	0.3609	0.0001	0.0220	0.0316

[a]Abbreviations: COD, center of domestication; MA, Middle American; Andean, Andean South America; GN, great northern; WK, white kidney; DRK, dark red kidney; SR, small red. [b]Mean separations were performed using Tukey's multiple-comparison tests. Means with the same letter are not significantly different at $p < 0.05$.

Coefficients of variation for IDF, SDF, and IDF+SDF among the methods were similar (Table 1). Coefficients of variation for oligosaccharides were similar for stachyose and total oligos in population II, but smaller for raffinose and TDF. In general, precision was similar when duplicate samples were used for each replicate than in those that utilized only one sample for each replicate as described above. Our results suggest that there is no need to utilize two duplicate samples per replicate to obtain a precision similar to that realized using duplicate samples.

The second modification involved changes to the HPLC procedure to generate detailed quantification of the individual oligosaccharides: raffinose, stachyose, and verbascose. The AOAC 2011.25 protocol suggests using HPLC with refractive index detection to quantify maltose and larger oligosaccharides; however, we implemented high-performance anion-exchange chromatography coupled with electrochemical detection (HPAE-PAD), a technique well suited for carbohydrates that permitted sample pretreatment simplicity, sensitivity to picomole levels, and improved peak resolution.[35−37]

The choice for the determination of protein content in the residues from the first and second filtrations was the Dumas combustion method rather than the Kjeldahl method.[38−41] The Dumas method utilizes combustion of the residue sample to convert all nitrogen forms to gaseous nitrogen oxides that are measured with a thermal conductivity detector.[39] The Kjeldahl method has been criticized because it is time-consuming and reliant on hazardous chemicals that pose a health risk and require specialized waste disposal; in contrast, the Dumas method minimizes the health and environmental risk.[38−41] The Dumas method also has an advantage in requiring a short analysis time of <5 min per sample and can be partially automated.[39,40] Marco et al. reported a 55% reduction in cost with the Dumas method compared to the Kjeldahl method in part because of the reduced labor time and waste disposal.[40] The Dumas method has also been cited for its repeatability and reproducibility, and it is recommended to replace the Kjeldahl combustion method.[38,40,41]

Variation in Fiber Content among Bean Entries. Variation of IDF, SDF, and IDF+SDF was observed among

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entries in both populations ($p < 0.05$) (Tables 2 and 3). The IDF fractions of IDF+SDF accounted for 69 and 70% in populations I and II, respectively. The observation that the IDF fraction accounts for the largest proportion of the IDF+SDF component of TDF corresponds with previous reports for legumes and common beans.[12,42] The levels of insoluble dietary fiber differed among entries in both populations ($p < 0.05$) (Tables 2 and 3). In population I, IDF ranged from 11.4 to 16.2% with a mean of 14.1% (Table 2). The mean IDF in population II was 13.7% and ranged from 12.4 to 14.9% (Table 3). The mean IDF among entries from the Middle American and Andean Centers of Domestication did not differ in either population.

Soluble dietary fiber also differed among entries in both populations ($p < 0.05$) (Tables 2 and 3). In population I, SDF ranged from 3.4 to 10.6% with a mean of 6.4% (Table 2). The mean SDF in population II was 5.9% and ranged from 3.3 to 7.7% (Table 3). The mean SDF was higher in the Andean entries than the Middle American entries only in population II.

Entries in population I differed ($p < 0.05$) for IDF+SDF and ranged from 16.8 to 23.2% with a mean of 20.5% (Table 2). In population I, Andean entries did not differ from Middle American entries for IDF+SDF. In population II, the IDF+SDF content also varied ($p < 0.05$) among entries and ranged from 17.3 to 22.5% with a mean of 19.6% (Table 3). Andean entries had higher mean IDF+SDF content than Middle American entries in population II ($p < 0.05$).

Oligosaccharide Content. We did not conduct a statistical analysis of oligosaccharides in population I because we used population I to develop the modified HPLC procedure and test the standard curves that were used for replicated analysis in population II. In population I, the total oligosaccharide content ranged from 2.56 to 4.65% with an overall mean of 3.44% (Table 2). Oligosaccharides were primarily comprised of stachyose, the level of which ranged from 2.29 to 3.98%, while the level of raffinose ranged from 0.23 to 0.73%. The verbascose content was very low or below quantifiable levels in most entries (data not shown); consequently, the levels are not included in our report. Very low verbascose levels in dry beans have been previously reported.[14,43]

The range of oligosaccharide content among entries in population II was similar to that among entries in population I. The total oligosaccharide content in population II ranged from 2.36 to 3.84% (Table 3). Entries differed ($p < 0.0001$) for total oligosaccharide content, and population II entries originating from the Middle American center of domestication (3.17%) had higher ($p < 0.05$) oligosaccharide content than entries from the Andean center of domestication (2.84%). The mean stachyose content among entries was 2.77% and ranged from 2.24 to 3.33% ($p < 0.0001$). The stachyose content did not differ between entry means from the Middle American and Andean centers of domestication (Table 3). Like observations in population I, stachyose also accounted for the majority of the total oligosaccharides in population II. The mean raffinose content in population II was 0.34% and ranged from 0.04 to 0.69%. Entries originating from the Andean center of domestication had lower ($p < 0.001$) raffinose content than those originating from the Middle American center of domestication (Table 3). The higher level of stachyose versus that of raffinose and the total oligosaccharide content observed in both populations correspond to observations previously reported.[15]

Total dietary fiber based on the AOAC 2011.25 method includes IDF, SDF, and oligosaccharide content.[29] This differs from previous methods that do not include resistant starch or oligosaccharides in the calculation for TDF, and as a result, the values obtained with AOAC 2009.01 and 2011.25 are slightly higher than values reported with previous methods.[16,34] Observed TDF values in population I ranged from 20.0 to 27.0% with a mean of 24.0% (Table 2). In population II, the TDF content among entries ranged from 20.6 to 25.7% ($p < 0.05$) with a mean of 22.7% (Table 3). Our results are in the range reported by McCleary et al., who reported TDF ranged from 20.9 to 27.3% in light red kidney beans using the AOAC 2009.01 method.[16,18] Entries from the Andean center of domestication had a higher mean TDF content than Middle American entries ($p < 0.05$) in population II.

Implications of Results and Future Directions. The ranges of observed values for IDF, SDF, oligosaccharide, and TDF content among entries in this study suggest that genetic variation exists among cultivars/lines of dry edible bean. Furthermore, the range of variation for these traits observed in two separate diverse populations of dry edible beans further indicates that there is adequate genetic diversity to allow selection for these traits in segregating breeding populations. In both populations, the mean IDF did not differ between Middle American and Andean entries; however, SDF and IDF+SDF were higher in Andean germplasm for population II. Conversely, the total oligosaccharide content was higher in Middle American entries than Andean entries in population II. These results suggest that Andean beans tend to have higher levels of beneficial dietary fiber (IDF+SDF) and lower levels of oligosaccharides that are associated with digestive problems. These results suggest that germplasm originating from the Andean center of domestication would be a good source of breeding high-fiber, low-oligosaccharide beans that are better tolerated in the human diet. Additional research is needed to investigate the role that the environment plays in determining the content of TDF and oligosaccharides in beans.

Overall, our results favorably support the use of the Total Integrated Fiber Assay AOAC 2011.25 method for the quantification of IDF, SDF, raffinose, stachyose, and TDF content in dry edible bean. The modifications made to the AOAC 2011.25 method in this study improve the efficiency for analyzing large numbers of samples that should allow it to become an important tool in our bean breeding program. The inclusion of a simple and accurate method for measurement of IDF, SDF, oligosaccharides, and TDF content makes the method an ideal choice for dry edible beans.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: mark.brick@colostate.edu. Phone: (970) 491-6551. Fax: (970) 491-0564.

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