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DETERMINATION OF DIETARY FIBER, AND TOTAL AND INDIGESTIBLE STARCH AND PROTEIN IN A SELECTED SAMPLE OF DRY BEAN (*Phaseolus vulgaris* L.) GENOTYPES

presented by

Maria Teresa Ospina

has been accepted towards fulfillment
of the requirements for

Master degree in Plant Breeding and Genetics

George L. Hosfield
Major professor

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DETERMINATION OF DIETARY FIBER, AND TOTAL AND INDIGESTIBLE STARCH AND PROTEIN IN A SELECTED SAMPLE OF DRY BEAN (Phaseolus vulgaris L.) GENOTYPES

By

Maria Teresa Ospina

AN ABSTRACT OF A DISSERTATION

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ABSTRACT

DETERMINATION OF DIETARY FIBER, AND TOTAL AND INDIGESTIBLE STARCH AND PROTEIN IN A SELECTED SAMPLE OF DRY BEAN (Phaseolus vulgaris L.) GENOTYPES

By

Maria Teresa Ospina

Dry seeds of common bean (Phaseolus vulgaris L.) are considered hard to digest and often cause gastrointestinal discomfort. Carbohydrates and protein contribute to this distress. Dry bean also has low starch digestibility and its incomplete digestion and absorption in the small intestine leads to fermentation after it enters the colon. In the bean sample preparation the particle size of cook and raw beans was comparable by grinding the freeze-dried samples so they would pass through a 40 and 60-mesh screen, respectively. In order to establish variability among different bean genotypes, determination of indigestible starch was performed on forty-one different bean genotypes, and two processing technologies — cooked and raw beans. In the determination of total dietary fiber, an indigestible residue is obtained. Statistics were used to declare significance for the variables indigestible residue, and indigestible starch. Cooked beans had higher amounts of indigestible residue and indigestible starch than raw beans. Determinations were also made for indigestible protein. In most of the genotypes, higher mean values of indigestible protein were obtained in cooked bean samples than raw bean samples. Results indicated that cooking beans increases the amount of indigestible starch and probably indigestible protein when compared with raw bean seeds.

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GENERAL INTRODUCTION

Dry seeds of common bean (Phaseolus vulgaris L.) have contributed essential protein, carbohydrates, vitamins, and minerals to human diets for 8 millennia. In general, bean seeds contain about twice the amount of protein found in cereals. Bean seed protein is rich in the amino acid lysine but deficient in sulfur-containing amino acids. Cereal grain proteins, on the other hand, are rich in sulfur-containing amino acids but deficient in lysine. Thus, bean protein and cereal protein complement one another's amino acid deficiency; and, thus, can provide a more utilizable protein to human diets than if each were consumed individually (Bressani, 1973).

In the new world there is botanical, archeological, and biochemical evidence that beans were domesticated in the Americas (Debouck *et al.*, 1988) about 7,000 years ago (Kaplan, 1965). Wild bean accessions were found widely distributed, from Chihuahua in Mexico [reported by Nabhan (1985)], to San Luis in Argentina [reported by Burkart and Brucher (1953), Debouck, *et al.*, 1988]. Archeological evidence demonstrates that the oldest bean plants were found in Jujuy, Argentina, Ancash, Peru, and Tehuacan and Puebla, Mexico. The wide distribution of beans in the American hemisphere shows that the bean plant can readily adapt to different environments.

Phaseolin, the most important storage protein in P. vulgaris, was found to be a useful marker for evolutionary studies (Gepts, 1988). This usefulness is the result of phaseolin's molecular complexity, which can be detected by its migration through an

electric field. The process by which charged particles move through a stationary liquid under the influence of an electric field is known as electrophoresis. Because of its complexity each phaseolin type is most likely unique and can, therefore, be used to trace the evolutionary origin of common bean genotypes (Gepts, 1984 and 1988). Considering their electrophoretic profiles, different phaseolin types have been identified. The type of phaseolin found in wild bean populations clearly showed the pattern of bean distribution in the Americas. Beans with the M and S type phaseolin were found in Mexico, Guatemala, and Costa Rica. Colombia was considered a transition zone for phaseolin type because CH and B type phaseolins were found. Peru, Bolivia, and Argentina had I, T, C, K, H, and J types of phaseolin.

Years after the discovery of the New World by Columbus in 1492, beans were widely cultivated in Western Europe. Common bean reached continental Europe between the early sixteenth and seventeenth centuries and England by 1594 [Kueneman *et al.*, 1978 (cited by Evans, 1976)]. From Western Europe, beans were distributed to the Southeast of Europe, Iran, India, and some places in Asia and Africa. Most of the bean varieties consumed in Europe were introduced there from the southern Andean Region. Beans were also brought back to the Americas, especially to Argentina and USA by Spanish explorers (Gepts *et al.*, 1988). Seeds of P. vulgaris were transported from Brazil to Africa by sailors engaged in the slave trade.

On a weight basis dry beans contain about 63% total carbohydrate, 22% crude protein, 9% water, 4% ash, and 2% fat (Watt and Merril , 1963). The carbohydrate fraction of bean seeds is made up of dietary fiber which is about 17%, starch about 40%,

and sugars about 6%. Sugars found in beans are sucrose (2.8%), raffinose (0.4%), stachyose (2.9%), and about 1% of other hexoses and pentoses (Tomkinson, 1986). Crude fiber is considered an old term and its use is being discontinued because the method of analysis that includes extraction of the plant material first with acid and then with alkali, recovers only variable portions of cellulose, hemicelluloses, and lignin present in dietary fiber (Spiller, 1986). The definition of dietary fiber includes cellulose, hemicellulose, pectin, lignin, and intracellular polysaccharides such as gums, mucilages, and starch that is not digested by mammalian digestive enzymes (Trowel *et al.*, 1976).

Dry beans are considered hard to digest and often cause gastrointestinal discomfort. Many people experience flatulence, cramps, and diarrhea after eating beans (Fleming, 1981). Both, carbohydrates and protein contribute to gastrointestinal discomfort. Nutritionists commonly accept the fact that beans have low protein digestibility, because high levels of nitrogen are lost when beans are consumed. Beans also have low starch digestibility with both intrinsic factors and cooking method contributing to this phenomenon (Hellendorn, 1969). Englyst *et al.* (1985) and Cummings (1983) have shown that incomplete digestion and absorption of starch in the small intestine lead to fermentation of the undigested starch after it enters the colon.

Since the 1960s, research has shown that the nature of starch (amylose and amylopectin) is the cause of its low digestibility in a wide range of foods (Borchers, 1961; Sandstedt *et al.*, 1962). Thorne *et al.*, (1983) showed that amylose and amylopectin differed in biochemical reaction to enzyme digestion. Recently, Chung (1996) suggested that starch indigestibility in P. vulgaris was mainly due to the crystallization of cell walls

in the cotyledon. The crystallization apparently encapsulates the starch granules; thus, rendering them inaccessible to attack by digestive enzymes (Chung, 1996).

Starch indigestibility in dry bean may be under genetic control. Murphy (1973) suggested genetic selection for improved starch digestibility might lead to beans with reduced flatulence. The bean variety 'Pike's Jacob's Cattle' was reported to be a gasless variety. One of its parents was 'Jacob's Cattle,' a reported gasless variety, and the other parent was a Black Mexican variety. Evaluation of human intestinal gas formation after consumption of 'Pike's Jacob's Cattle' showed that the amount of intestinal gas evolved was less than what one would expect among cultivars of P. vulgaris. Murphy (1973) concluded that flatulence appeared to be under genetic control and can be reduced by genetic selection. From the foregoing, one can formulate several questions regarding indigestibility and flatulence from eating beans. Is it possible that some bean varieties are more digestible than others because of differences in starch and protein quantity and physical-chemical characteristics? Since the majority of the macromolecular content of a bean seed is starch, how digestible is starch? Is starch digestibility under genetic control? Are varietal differences in starch digestibility large enough to be altered through selection? Does cooking make starch more indigestible than the starch in raw beans?

The answers to the proposed questions formulated the basis for the present study. The research objectives were to: 1.) Develop suitable methodology for measuring indigestible components in bean seeds, 2.) Determine if there is variability of indigestible components, especially starches in bean seeds, and 3.) Measure the range of variability for indigestible components in bean seeds among a diverse group of genetic stocks.

GENERAL LITERATURE REVIEW

Nutrient Composition of Dry Beans

Carbohydrates

The carbohydrate content in dry bean seeds is about 60% on a dry basis (Kay, 1979; Price *et al.*, 1988). Monosaccharides, oligosaccharides, polysaccharides comprise the carbohydrates in beans. The largest portion of the bean carbohydrate fraction is starch, a storage polysaccharide. In the literature reported values for starch content in dry beans vary from 24 to 56% due to differences among cultivars and analytical procedures (Cerning *et al.*, 1975; Lai *et al.*, 1979; Reddy *et al.*, 1984).

Starch.

Starch is composed of two different glucose polymers — amylose and amylopectin, and in dry bean seeds, starch is organized into semicrystalline granules. Amylose is a long chain of glucose units linked by 1,4-∝-D-glucopyranosidic bonds. Amylopectin is a branched structure of glucose molecules with interconnecting 1,6-∝-D-glucopyranosidic bonds (Dreher *et al.*, 1984; Whistler *et al.*, 1984). The molecular weight of amylopectin is higher than that of amylose. Unlike amylopectin, amylose has glucose chains with strong (1-4) bonds, which make it difficult for enzymatic digestion (Thorne *et al.*, 1983).

Dietary Fiber.

Dietary fiber is composed of those portions of plant cells that are not digested by

human enzymes, such as polysaccharides (cellulose, hemicellulose, oligosaccharides, pectins, gums, waxes) and lignin (Trowell *et al.*, 1976). Starch was also found not to be totally digested by alimentary enzymes. This starch is called resistant starch (Englyst *et al.*, 1987). Two types of dietary fiber are distinguished -- soluble and insoluble. The content of dietary fiber in P. vulgaris ranges from 17.5 to 20%; thus, dry beans are considered a good source of dietary fiber (Prosky *et al.*, 1985). The type and amount of fiber consumed determines the variation of function of dietary fiber in the gastrointestinal tract and utilization of nutrients. Lignin, cellulose, and hemicellulose are considered insoluble forms of dietary fiber. Pectin, gums, mucilages, and starch constitute the soluble dietary fiber. Water-insoluble dietary fiber affects the passage of materials in the gastrointestinal tract and reduces the absorption of nutrients. Water-soluble dietary fiber affects the metabolism of carbohydrates and lipids (Muñoz, 1986). The plant species, its age, and growth conditions determine properties and metabolic effects of dietary fiber. This may explain some of the variation in experimental results obtained by different researchers, even when using the same technique. Chemical methodologies allow estimation of total, insoluble and soluble dietary fiber (Lee *et al.*, 1992).

Proteins

Protein content in legumes is among the highest found in the plant kingdom and is considerably greater than in the cereals. Bean protein ranges from 19 to 30% with an average of 25% (Bressani, 1973; Tomkinsom, 1986). Cereals contain about 9 to 18% protein. Oat (Avena sativa L.) is the cereal with the highest protein content, about 18%. True protein digestibility of legumes varies from 59.5% in *Cajanus* to 93.9% in *Pisum*

sativum, and evaluations in P. vulgaris showed variations among 76.8 to 84.1% (Jaffe, 1973; Bressani, 1973).

Variability for protein content in P. vulgaris, as well as other nutrients and amino acids, is affected by variety and location (Bressani, 1973). Bean proteins have higher amounts of lysine and threonine than methionine and cystine. Salt-soluble globulin —a storage protein of P. vulgaris— can be resistant to enzymes that may account for the low digestibility of protein (Seidl *et al.*, 1969).

Lipids

Most legumes are low in total lipid content. Lipids in legumes are composed of neutral lipids (e.g., triacylglycerol), phospholipids (e.g., phosphatidycholine), and glycolipids (esterified steryl glucoside). Neutral lipids are the predominant class of lipids that vary from 32 to 51%, of the total lipid fraction. Phospholipids constitute 23 to 38%, and glycolipids 8 to 12% of total lipids (Salunkhe *et al.*, 1989). The content of lipids increases 20% during seed maturation. Neutral lipid content increases faster than phospholipids or glycolipids during this stage of the plant growth cycle. Phospholipids and glycolipids are part of the seed membrane (Salunkhe *et al.*, 1989). Components of the fatty acids such as palmitic, oleic, linoleic, and linolenic acids were found in neutral lipids, phospholipids, and glycolipids of legumes (Mahadevappa *et al.*, 1978). The content of lipids in beans is generally less than 2.0% (Watt *et al.*, 1963; Tomkinson, 1986). Drumm *et al.*, (1989) showed a 1.8 to 2.6 g per 100 g dry weight lipid content among four bean market classes (navy, dark red kidney, pinto, and black turtle soup).

Drumm *et al.*, (1989) also found that linolenic acid, an unsaturated fatty acid and palmitic and stearic acids; both saturated fatty acids, were the major fatty acids in these beans. Significant differences were found among the four bean classes for each lipid component: neutral lipids, phospholipids, and glycolipids (Drumm *et al.*, (1989).

Minerals

The mineral content of a substance is found by heating at 500°C until all that remains is ash. Legumes are a good source of minerals like calcium, iron, magnesium, copper, zinc, and potassium (Salunkhe *et al.*, 1985). The amount of calcium found in legumes is higher than that found in cereals (Douty *et al.*, 1982). The content of ash (minerals) in P. vulgaris varies from 3.5 to 4.1% (Kay, 1979). Minerals and their association with other components can be altered with processing of food. Mineral availability can be inhibited by dietary components such as proteins and complex carbohydrates.

Vitamins

Legumes are sources of thiamin, niacin, riboflavin, and folic acid (Salunkhe, 1989). Kasper (1986) stated that there are two possibilities of how dietary fiber substances effect vitamin metabolism. First, dietary fiber can effect ingested vitamins, by reducing their absorption from the intestine. Studies with humans and animals have demonstrated that the absorption of vitamin A, carotene, and vitamins B_2, B_6, B_{12} is influenced when lignin, pectin, and cellulose are added to the diet. Second, intestinal vitamin synthesis is effected by the interaction between dietary fiber and vitamin

metabolism. Experiments with animals showed that carbohydrates, which are difficult to digest, such as raw potato starch, pectin, and cellulose stimulate the synthesis of B vitamins and vitamin K in the intestine (Kasper, 1986).

Dry Bean Digestibility

Sample Preparation

Drying of samples before grinding is important to assure consistency in results. Particle size may also affect the results of digestibility evaluations (Horvath *et al.*, 1986). When drying bean flour above 60°C or in a microwave oven, in vitro values of dietary fiber and lignin increased (Heller *et al.*, 1977); in the same way, when particle size decreased, dietary fiber decreased as well as other fractions varying non-uniformly (Heller *et al.*, 1977). Heller *et al.* (1977) concluded that the optimum way to dry samples of food for grinding was by freeze-drying.

There are different methods in sample preparation for studies of indigestible components of food that mimic human digestion. Food can be chewed before enzyme digestions (Muir *et al.*, 1993) or it can be pulverized through a mincer with 0.9 cm diameter holes (Englyst *et al.*, 1992). The mincing methodology to prepare food samples for digestibility studies gave more consistent data than when the samples were chewed (Englyst *et al.*, 1992). Although chewing of samples has its limitations in determining indigestible residue in food, this method may be more appropriate than milling or homogenizing samples (Muir *et al.*, 1994).

Starch Digestibility

Starch digestion occurs through the action of salivary and pancreatic α-amylases, which produce maltose and α-dextrins. Maltose in turn is catabolized by intestinal glucosidases that produce glucose (Manners, 1978). Digestibility in dry bean depends on the condition of the seed and on cooking time. Hellendoorn (1973) stated that starch digestibility in raw bean increases 20% after soaking, and can increase rapidly up to 95% after one hour of cooking under pressure. Chung (1996) found that when beans were cooked for a long time, the cell walls of cotyledons become rigid, encapsulating the granules; thus, producing a barrier to enzymatic hydrolysis. The rate of digestion of starch and sugars in beans is determined by the physical form of the food and not by the large complex polymers of starches (Wahlquist *et al.*, 1978; Jenkins *et al.*, 1987). Availability of glucosidic bonds of starch to enzyme attack can determine the starch digestion rate. Starch is incompletely digested in the small intestine (Englyst *et al.*, 1992). The amount of starch that escapes digestion in the small intestine varies depending on the food preparation, the starch form, and the method of analysis. Undigested starch can vary from less than one percent to more than 20% (Schweizer, *et al.*, 1990). Starch that escapes digestion within the small intestine, enters the colon where it serves as an energy source for colonic bacteria (Cummings, 1983). Digestion of starch can be altered by dietary fiber components, such as cellulose, hemicellulose, and lignin. The protease and amylase inhibitors in P. vulgaris can reduce starch digestion, as well as the lectin residues present after the cooking process. On the other hand, starch digestion may interfere with

the availability of proteins, lipids, minerals, and vitamins.

Indigestible components, such as dietary fiber, effect the functioning of the digestive processes in humans by decreasing the availability of nutrients. Nutrient assimilation requires movement of food through the digestive tract, enzymatic hydrolysis of complex nutrients, and absorption of smaller compounds in the small intestine. Schneeman *et al.,* (1986) concluded from in vitro experiments that dietary fiber restrains digestive enzyme activity and may slow starch or carbohydrate hydrolysis. Any undigested dietary constituent that is not digested in the small intestine can act as a substrate for fermentation in the large intestine (Fleming, 1986), and dietary fiber is considered a fermentable substrate. Beans are a high fiber food that often cause quantities of intestinal gas to be produced (Calloway et al., 1971).

Indigestible Starch

Several techniques reported by Johansson *et al.*, (1984); Holm *et al.*, (1986); Tovar *et al.*, (1990); Englyst *et al.*, (1992), and Muir *et al.,* (1993) for determination of indigestible starch utilized the same general principle, which is the digestion of starch by the enzyme α-amylase producing dextrins and maltose. These components are removed by filtration or centrifugation. The remaining starch (resistant starch) is determined by solubilizing the starch with 2 M KOH, and digesting the resistant starch to form glucose with amyloglucosidase. Glucose is quantified to estimate the equivalent amount of resistant starch.

Breeding for Digestibility in Dry Beans

Since the 1960s, the improvement of food quality in dry bean has been an important plant-breeding objective. Breeders have used various approaches to raise the protein content, remove anti-nutritional compounds, improve the amino acid balance and digestibility, and lower flatulence in beans. However little breeding work has been done to improve digestibility of carbohydrates including starch. Murphy (1973) mentioned that genetic selection might be feasible in dry bean to lower flatulence of which undigested starch is a large contributor (Tomkinson, 1986). There is a paucity of information on the genetic variability of indigestible components in beans including protein and starch to provide guidance for the breeder in plant improvement programs.

STUDY I

METHODOLOGY TO DETERMINE INDIGESTIBLE STARCH IN DRY BEANS (Phaseolus vulgaris L.)

INTRODUCTION

In dry bean (Phaseolus vulgaris L.) the improvement of digestibility and food quality of cooked seeds is an important research objective for food technologists, nutritionists, and plant breeders. The incomplete digestion and absorption of food in the small intestine lead to the fermentation of the undigested chemical constituents after they enter the colon. Seed proteins and carbohydrates are the main indigestible components in dry bean. Of the carbohydrate fraction, starch can be a significant source for fermentation in the large intestine (Tovar *et al.*, 1990). Indigestible starch in dry beans causes gastrointestinal discomfort including flatulence, cramps, and diarrhea (Fleming, 1981).

Persons in the food industry and consumers generally agree that dry bean consumption would increase if the digestibility of this food crop could be improved and the associated gastrointestinal difficulties ameliorated. There is anecdotal ('Jacob's Cattle' bean) and experimental evidence (Murphy, 1973) that genetic variability exists in dry beans for flatulence and related gastrointestinal discomfort. However, the flatulence described by Murphy (1973) was probably mostly due to α-oligosacharrides.

Indigestible starch can be determined in vitro in a step-wise fashion that includes the digestion of bean flour with enzymes, solubilization of resistant starch, and the

enzymatic degradation of resistant starch to glucose. Enzymatic digestions can be preformed using the total dietary fiber (TDF) assay suggested by Lee *et al.* (1992). In this assay, three sequential enzymatic digestions are carried out by α-amylase, protease, and amyloglucosidase, where each enzyme has a specific function in the process of digestion; the resulting TDF is also called indigestible residue (IR). The indigestible starch remaining in TDF is determined by solubilizing the indigestible starch with a base (e.g., 2N NaOH), and digesting the starch with amyloglucosidase to produce glucose. Finally, the amount of glucose is determined and equated to the quantity of indigestible starch in the sample.

Nutrient determinations in most foods are determined on the raw product. However, dry beans are generally soaked and must be cooked to render the seeds palatable, inactivate heat labile anti-nutrients, and permit the digestion and assimilation of protein and starch (Deshpande *et al.*, 1983). Wassimi *et al.*, (1988) found a significant amount of protein loss in soaked and cooked beans. Moreover, the variation in protein loss found in the study by Wassimi *et al.* (1988), demonstrated that consideration other than the nutrient content of raw beans is important. Because of the possibility of nutrient loss in beans during their preparation for eating, it would be logical to determine indigestible starch on both raw and cooked beans. Moreover, Chung (1996) found that when beans are cooked, the cell walls of cotyledons become rigid and encapsulate starch granules. Chung's (1996) research provides evidence that cooked bean starch may be less digestible than raw bean starch.

For analysis of food components, samples should be homogeneous before subsampling. A lack of homogeneity between subsamples may lead to a large sampling error in the experiment. Freeze-drying before comminution is recommended for sample preparation (Heller *et al.*, 1977) because freeze-drying reduces undesirable changes in the sample. The comminution of a food may be accomplished either by chewing or milling. The chewing technique is a natural method of food comminution because it gives a better approximation of the physiological digestive process than does the milling method. Although chewing the sample may be more appropriate from a physiological stand point, milling and homogenizing are preferred to obtain consistent results. Milling and homogenizing a sample may underestimate the true resistant starch; however, the data would be consistent and reproducible from laboratory to laboratory.

In many cases the particle size of the food sample should be reduced for analysis (Horwitz, 1988) and for homogenization. Mills are used to reduce food into small particles, the size of which simulates food in the intestinal tract. Mills are fitted with screens that control the size of particles. For dried foods, particles of 20-mesh screen size should be used for determining moisture, total protein, or mineral content (Proctor *et al.*, 1998). Particles for lipids and carbohydrate analysis should be of a size to pass through a 40-mesh screen (Proctor *et al.*, 1998). In preparing bean samples to evaluate digestibility, one must ensure that the particle sizes between raw and cooked samples are comparable. The importance of particle size in digestion studies was reported by Cummings (1986), who found that when the same source of fiber was fed at two different particle sizes, greater stool output occurred with the larger particle size. The result of Cumming's work

(1986) suggests that larger particles are more slowly digested than smaller ones. The hypothesis that particle sizes between raw and cooked beans are different is tenable. Soaking and cooking cause structural changes in cells that most likely effect the size of particles resulting after beans are ground.

Because of the importance of indigestible starch in human nutrition and consumer acceptance of beans as a food, this study was undertaken. The specific objective was to develop an appropriate methodology to determine indigestible starch in dry bean that was accurate, repeatable, and useful to plant breeders.

MATERIALS AND METHODS

Plant Materials

'Montcalm' dark red kidney bean was the experimental material on which particle size distribution and indigestible starch determinations were made. 'Montcalm' has a typical kidney shaped seed that weighs approximately 65 g · 100 seed^{-1}. 'Montcalm' was grown in the nursery during the summer of 1996 on a M^{c}Bride Sandy Loam (Coarse – loamy, mixed, frigid Alfic Fragiothods) soil at the Montcalm Research Farm near Stanton, MI. Seeds were harvested from mature plants, cleaned of plant and soil debris and stored in Kraft® paper bags at 16°C until laboratory evaluations were made.

Sample Preparation

Four treatments were imposed on 'Montcalm' dark red kidney bean to ascertain the effects of sample preparation and particle size on the amounts of TDF and indigestible starch.

Treatment 1- cooked beans. One hundred-gram samples of beans were soaked in distilled water in a beaker for 12 hours. The beans were drained and placed into a 1.0-L beaker to which 500-g of distilled water was added. The beakers were placed on a hot plate and brought to a slow boil. Beans were cooked until eating soft which took approximately one hour. Eating soft was determined tactilely by placing individual beans between the thumb and index finger and lightly squeezing the beans. Beans that ruptured readily upon the light compression force and had a paste-like consistency were

considered eating soft. After the beans were determined eating soft, the water remaining in the beaker was drained, and the beans cooled and lyophilized in a Virtis-Genesis 12EL freeze dryer.

Treatment 2- raw beans. One hundred-gram samples of raw beans were used without soaking and lyophilizing.

Treatment 3- soaked raw beans. One hundred-gram samples of beans were soaked for 12 hours in a beaker containing 500-g of distilled water. After the beans were soaked, the beans were drained, dried, and lyophilized in a Virtis-Genesis 12EL freeze dryer.

Treatment 4- raw and humidified beans. One hundred gram samples of beans were placed in a humidity chamber and held at 40°C until beans gained about 50% of their initial weight. The beans were lyophilized in a Virtis-Genesis 12EL freeze dryer.

Particle Size Distribution

Bean samples from each treatment, i.e., cooked, raw, raw + soaked, and raw + humidified were ground to a flour using a Wiley mill fitted with a 40-mesh screen. After the samples were ground, the particle size distribution of the flour was determined on an instrument containing screens that were layered from top to bottom and with the following mesh sizes: 26, 40, 48, 54, 74, 94, and 120. The ground bean flour from each sample from each treatment was weighed and placed on top of the 26-mesh screen (top most screen). The instrument was shaken for 10 minutes to allow different sized particles to percolate through the various screens. The residues were then collected from each

screen and weighed. The flour that passed through the 120-mesh screen was collected in the pan at the bottom of the screens. The amount of a bean flour sample collected in each of the mesh screens from each of the treatments was reported as a percentage of the initial weight of bean flour in grams placed in the 26-mesh (top) screen.

Bean flour samples from the raw and raw + soaked treatments were ground with a Wiley mill fitted with a 60-mesh screen, and the particle size distribution determined in the same way as for the samples ground through the 40-mesh screen described above.

Total Dietary Fiber (TDF) Assay

The determination of indigestible starch in beans includes digestions with enzymes, solubilization of starch, and enzymatic degradation to glucose. Enzymatic digestions were performed using the Total Dietary Fiber (TDF) assay (Fig. 1) suggested by Lee *et al.,* (1992). In this assay, three sequential enzymatic digestions were carried out by a-amylase, protease, and amyloglucosidase, where each enzyme had a specific function in the process of digestion. The indigestible starch remaining in TDF was solubilized with 2 N NaOH, digested with amyloglucosidease, and the glucose that resulted from the enzyme digestion was determined spectrophotometrically.

Total dietary fiber (TDF) was determined on the cooked, raw, and raw + soaked treatments (Fig. 1). The raw + humidified treatment was eliminated from further consideration because the particle size distribution profiles were greatly discordant from the raw and cooked particle size distributions. The amount of bean sample used for each assay was 1.000 ± 0.005 g. Duplicate 1.000 ± 0.005 g samples of each of the three

preparation treatments were placed into a 400-ml beaker to which 40 ml of a 2-(N-Morfolino) ethanesulfonic acid/Tris (hydroxymethyl) aminomethane (MES/TRIS) buffer pH 8.2 solution was added and stirred at low speed until all clumps disappeared. Heat-stable α-amylase solution (50 µl) was added to the solution while stirring. Beakers were covered with aluminum foil and placed into a water bath at 95°C with constant shaking for fifteen minutes. The beakers were then uncovered, placed in a water bath at 60°C. Distilled water (10 ml) was added to each beaker to rinse the beaker walls. A protease solution (100 µl) was added to each beaker and the samples incubated at 60°C with constant agitation for 30 minutes. A 5 ml solution 0.561N hydrochloric acid (HCL) was added to each beaker and pH was adjusted to a range of 4.0 - 4.7 using either 1N HCL or 1N sodium hydroxide (NaOH). Amyloglucosidase solution (300 µl) was added to each beaker while stirring. Beakers were covered with aluminum foil and placed in a water bath to incubate at 60°C for 30 minutes. A 225 ml of a 95% ethyl alcohol solution, which was brought to 60°C, was added to each digested sample. Samples were left to stand for one hour at room temperature to precipitate. The samples were filtered under vacuum through crucibles with sintered glass. The residue remaining in each crucible was washed twice, first with 78% alcohol, then 95% alcohol, and finally acetone (15 ml each) during the filtering process. The residue remaining in the crucibles was considered the dietary fiber (indigestible residue). This residue was left in the crucibles, which were dried in an oven at 105°C overnight. After the crucibles were removed from the oven they were cooled in dessicators and weighed to the nearest 0.1 mg. The total dietary fiber

residue from each sample was used to determine the amount of indigestible starch.

Determination of Indigestible Starch

The residue of each sample obtained from the TDF assay was gently scraped from the crucible and transferred into beakers and weighed. A 2N NaOH solution (10-ml) was added to each beaker while stirring to solubilize the starch (Fig. 1). When the residue in the beakers was completely mixed, beakers were allowed to stand at room temperature for one hour. Ten ml of a 1M (pH 4.5) acetate buffer was added to each beaker and the pH of the solution was adjusted to 4.5 using 6N HCL. Water was added to each beaker to bring the net weight to 100 g, and 400 µl of the solution was pipetted into each of four small screw-capped tubes (four tubes per beaker). Three units of amyloglucosidase (from Aspergillus niger, Boehringer Mannheim) in 100 ul of 0.1 M acetate buffer (pH 4.5) was added to each screw-capped tube. The tubes were incubated at 55°C overnight. Glucose was determined from 40 µl of the solution from each screw-capped tube.

Glucose Assay

A working glucose standard was prepared in duplicate by pipetting into test tubes 10, 20, 30, and 40 µl of a standard solution containing 100 mg/dL of glucose. Distilled water was added to each test tube to bring each sample to a total volume of 40-µl. Duplicate reagent blanks of 40-µl of distilled water were used. One ml of the combined enzyme color reagent (SIGMA Kit No. 510A) containing Glucose Peroxidase (PGO) and o-Dianisidine dihydrochloride, was added to each test tube, mixed and incubated for 45 minutes in the dark at room temperature (Fig. 1). Samples were analyzed by

transferring 40 µl of the amyloglucosidase digest solution into test tubes containing 1 ml of the combined-enzyme color reagent. The tubes were mixed and incubated in the same way as the standards. Absorbance was read with a spectrophotometer at 450 nm using micro plastic cuvettes. Distilled water was used to zero the spectrophotometer. The four readings from each solution were averaged. Absorbance versus glucose concentration was used to calculate the slope and intercept of the glucose standard curve.

Calculations

A constant multiplication factor (mf) from dilutions in each of the three assays was calculated (Fig. 1, Footnote [1]). The factor mf1 represents the amount of residue used for the determination of resistant starch taken from the total residue weight (indigestible residue) during the total dietary fiber assay. Factor mf2 (Fig. 1, Footnote [2]) is for the 0.4 ml that was removed from beakers containing 100 ml of the solution during the assay of resistant starch determination. The factor mf3 (Fig. 1, Footnote [3]) represents the 40 µl taken from 500 µl of the solution during the amyloglucosidase hydrolysis of resistant starch. The 0.9 value is the equivalent to the weight of glucose as starch is being formed, since 0.1 is liberated as water when glucose molecules are added to the polymer.

The product between the amount of glucose (µg) found in 40 µl of the solution and the factors mf1, mf2, mf3, and 0.9, was taken as the amount of indigestible starch present in a bean flour sample.

BEAN SAMPLE PREPARATION

⇓

TOTAL DIETARY FIBER

(Removal of sugars and digestible starch and protein)

1.0 g bean sample

⇓

Heat-Stable α-Amylase
(Gelatinization and starch hydrolysis)

⇓

Protease
(Protein hydrolysis)

⇓

Amyloglucosidase
(Starch and dextrin hydrolysis)

⇓

Ethanol Precipitation, Filtration, Washes

⇓

Dry residue (total fiber) * (1)

⇓

INDIGESTIBLE STARCH DETERMINATION

Solubilization (2 N NaOH)

⇓

Brought to pH 4.5 and diluted to 100 ml

⇓

400 µl taken to be hydrolyzed * * (2)

⇓

Amyloglucosidase (100 µl)
(Digestion of resistant starch)

⇓

Incubation

⇓

40 µl taken for glucose analysis * * * (3)

⇓

GLUCOSE ANALYSIS

(Sample digestion)

⇓

Combine enzyme-color reagent

⇓

Determination of absorbance with Spectrophotometer

* (1) Step that corresponds to mf1; * * (2) Step that corresponds to mf2; * * * (3) Step that corresponds to mf3

Figure 1. Methodology to determine indigestible starch in dry beans.

RESULTS AND DISCUSSION

Particle size determinations

The flour from the cooked, raw, raw + soaked, and raw + humidified bean treatments had different particle sizes when they were ground with a Wiley mill fitted with a 40-mesh screen (Fig. 2). For ground beans that were cooked, about 90% of the particles were extremely fine and passed through the 120-mesh sieve. The remaining 10% of the sample was collected from the 74, 94, and 120 mesh-screens (Fig. 2A). Particles of the raw and raw + soaked bean treatments were larger than for the cooked bean treatment, and less than 1% of the particles passed through the 120-mesh screen (Figs. 2B and 2C). For these two-sample preparation methods, the majority of the flour particles were collected from the 74, and 94-mesh screens. About 6, 15, 33, 14, and 24% of the flour from the raw + humidified treatment were collected from the 48, 54, 74, 94, and 120-mesh screen, respectively (Fig. 2D).

Because the particle sizes of the raw and raw + soaked bean treatments were not comparable to the cooked bean samples, raw and raw + soaked beans were ground in a mill fitted with a 60-mesh screen. When the particle size distribution was determined on the raw bean treatment, only about 1% of the flour sample passed through the 94-mesh screen (Fig. 3A). When raw beans were soaked for 12 hours and freeze-dried before grinding (raw + soaked treatment), about 9% of the sample passed through the 94-mesh screen. About 52% of the flour from the raw + soaked treatment passed through the 120-mesh screen (Fig. 3B). Examination of data indicated that the cooked beans ground in a

mill fitted with a 40-mesh screen and raw + soaked beans ground in a mill fitted with a 60-mesh screen were more similar in particle size distribution (Figs. 2A and 3B) than the other profiles. The distribution of particle sizes from the humidified bean treatment did not follow the pattern of the other samples (Fig. 2D). For this reason this treatment was not used for the indigestible starch determinations. When raw beans (not soaked or humidified) were ground in mills with either a 40 or 60-mesh screen, frequent screen breakage occurred. However, raw beans that were soaked for 12-hours or humidified and freeze-dried rarely caused screens to break when the beans were ground. Soaking and freeze-drying of raw + soaked beans appeared to facilitate an easy grinding of the samples and essentially eliminated screen breakage. Although hardness tests were not conducted, the particles from the freeze-dried raw + soaked beans may have been more friable than particles from raw beans not soaked and freeze-dried. Alternatively, the smaller and more uniform particle sizes associated with ground raw beans that were soaked and freeze-dried before grinding may have exerted less force against the screen and, thus, caused less screen damage than the larger and less uniform particles that resulted when raw beans without soaking or freeze drying were ground.

Once a satisfactory method to ensure comparable particle sizes of raw and cooked beans was achieved, an analytical procedure to determine the amount of indigestible starch in a sample could be developed. After many preliminary experiments, the analytical procedure finally chosen (Fig. 1) enabled the direct and quantitative determination of TDF and indigestible starch on the same sample.

The amount of indigestible starch detected was 22.7% in the cooked bean sample

and 1.5% in the raw + soaked freeze-dried bean sample, when both samples were ground using a 60-mesh screen (Table 1).

Grinding beans and sieving the resultant flour through the appropriate screen to give raw and cooked beans similar particle sizes eliminated the problem of overestimating the amount of indigestible starch in raw beans compared to cooked beans. The importance of particle size in digestion studies was reported by Cummings (1986), who found that when the same source of fiber was fed at two different particle sizes, greater stool output occurred with the larger particle size. The result of Cumming's work (1986) suggests that larger particles are more slowly digested than smaller ones. When raw beans were ground and sieved through a 40-mesh screen the larger particles compared to the particles from cooked beans ground and sieved through the same 40-mesh screen were more slowly digested leading to an inflated value of indigestible starch. The accurate estimation of indigestible starch in dry bean should lessen errors in data and provide plant breeders with a protocol to screen breeding lines for starch digestibility with a high degree of precision.

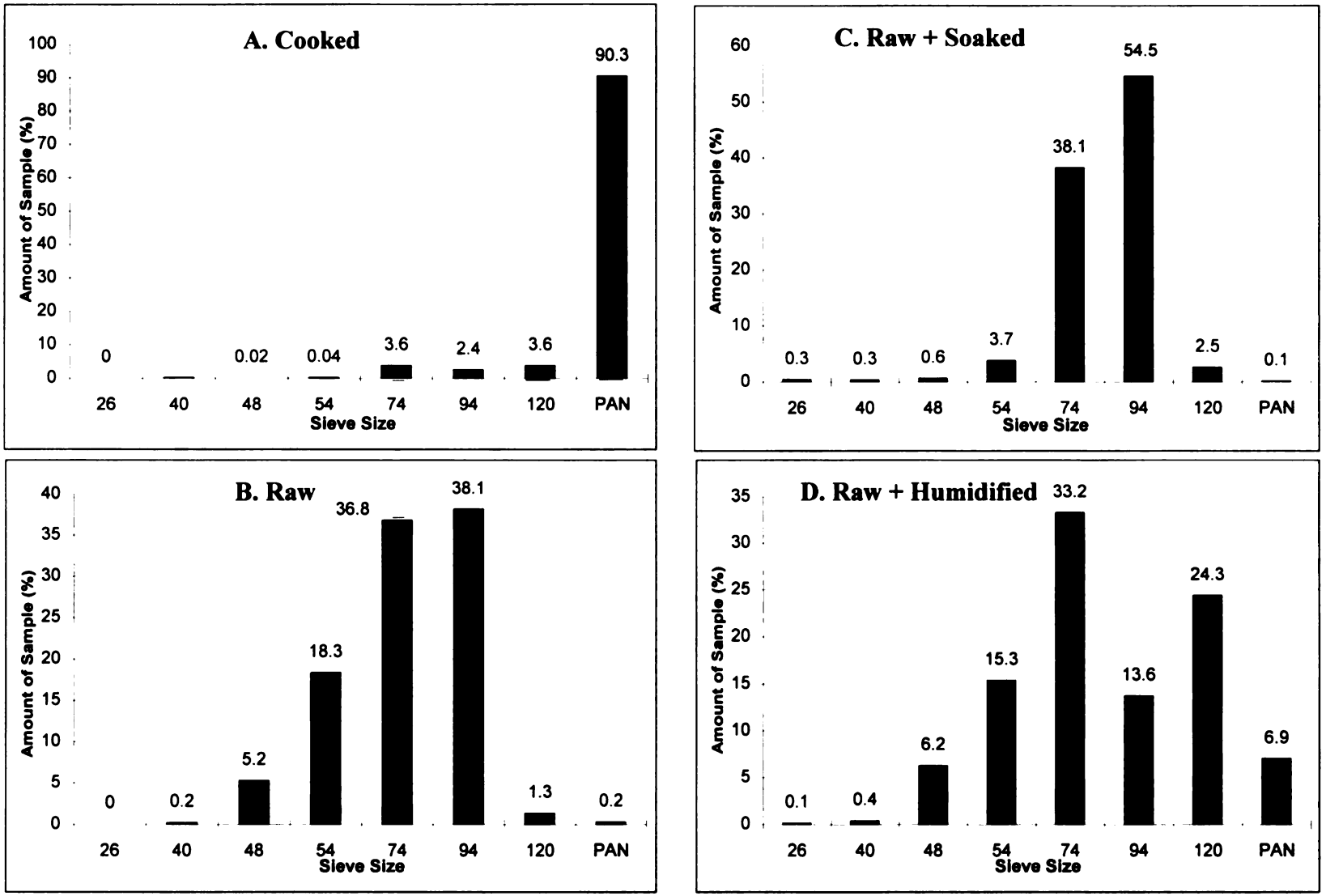
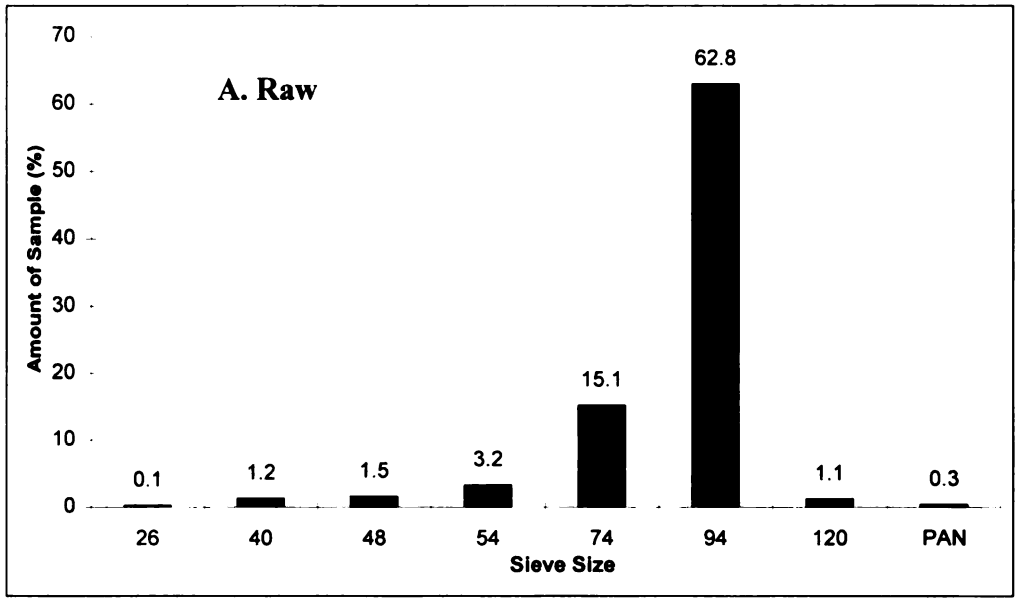


Figure 2. Particle Size Distribution in Cooked, Raw + Soaked, and Raw + Humidified Bean Samples Ground Through a 40-Mesh Screen.



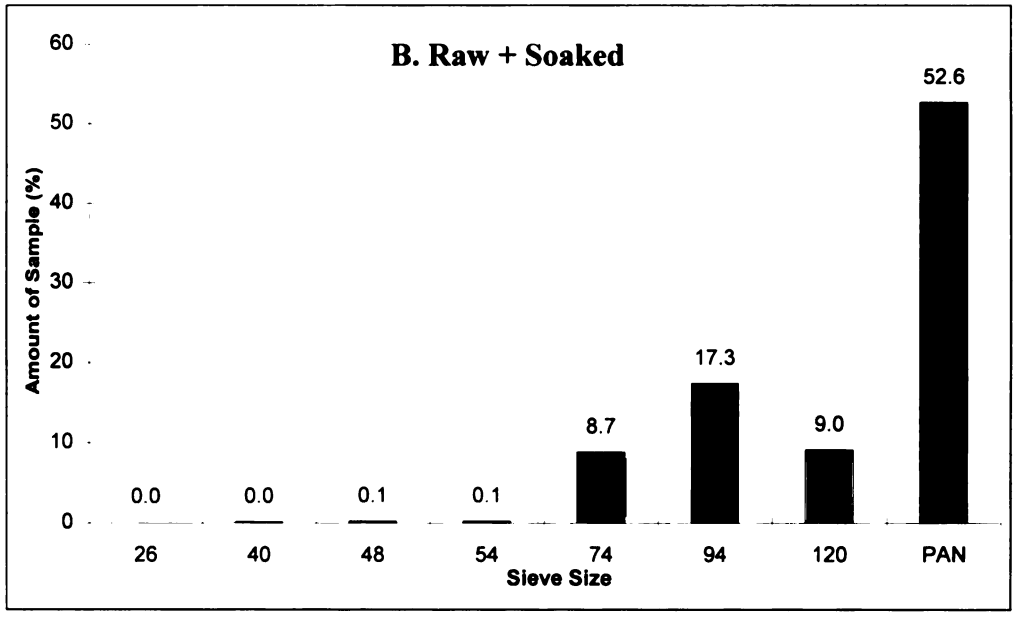


Figure 3. Particle Size Distribution in Raw and Raw + Soaked Bean Samples Ground Through a 60-Mesh Screen.

Table 1. Residue obtained from the Total Dietary Fiber (TDF) assay and indigestible starch (IS) calculated in bean sample treatments, cooked and raw, ground with 40 and 60-mesh screens.

	40-mesh screen		60-mesh screen	
Treatment	Total dietary Fiber (mg)	Indigestible Starch (mg/g of flour)	Total dietary fiber (mg)	Indigestible Starch (mg/g of flour)
Cooked	393.4	22.7	-	-
Raw*	330.8	13.6	238.7	2.7
Raw + soaked	251.9	2.0	195.6	1.5
Humidified	317.6	10.8	-	-

* Grinding raw beans through a 60-mesh screen was difficult because frequent screen breakage occurred.

STUDY II

DETERMINATION OF INDIGESTIBLE STARCH IN A SELECT SAMPLE OF DRY BEAN (Phaseolus vulgaris L.) GENOTYPES

INTRODUCTION

During most of the 20th century, crops used for human consumption have been improved through plant breeding for numerous characteristics of which those of high economic importance receiving the most attention. Yield and pest resistance are generally the major goals of breeding programs. Not with standing the importance of yield, breeders do not neglect food-quality improvements. Food quality includes characteristics that have a direct impact on human nutrition and those related to preparation for eating (culinary).

Breeding for a new trait of interest includes basic steps. First, the breeder must decide on a screening procedure. Second, germplasm is screened, and then useful genetic variability is determined. Finally, parents are selected for inheritance studies. The screening technique used must be accurate, reproducible, capable of dealing with small samples, and cost effective. In the evaluation of germplasm, adapted genotypes, elite lines, exotic lines, and introductions can be included.

Dry edible bean (Phaseolus vulgaris, L.) is an important food globally. Many developing nations depend on beans for nutrition, because often protein from animal sources is scarce and/or expensive. In addition to high protein, beans are a source of

vitamin B, iron, complex carbohydrates, and soluble fiber. In developed countries such as the U.S., United Kingdom, and Canada, replacing high meat and high fat diets with beans may lower cholesterol, and may be associated with a lower incidence of stroke, heart disease, and colon cancer. With changes in nutritional guidelines and culinary habits one may anticipate an increase in the domestic consumption of dry beans. Annual consumption of dry beans in the U.S. has increased from 5.4 to 9 lbs. since 1980 (Lucier, 1999). This increase in consumption may be the result of the recognition that including beans in the diet may confer important health benefits.

Despite wide acknowledgement of the crop's nutritional superiority compared to cereals, root crops, and other vegetables, beans are underutilized partly because they can cause gastrointestinal discomfort after they are ingested. The major discomforts associated with eating beans are cramps, flatulence and diarrhea (Tovar *et al*, 1990). There is anecdotal evidence that some beans are easier to digest than others and do not cause gastrointestinal problems.

The problems of low digestibility of dry bean and its physiological effects are of interest to nutritionists and plant breeders. Indigestible starch in beans may be the greatest contributor to flatulence and related gastrointestinal discomfort among the seed constituents. Chung (1996), stated that crystallization of the cell walls in the cotyledon acts as a physical barrier to enzyme hydrolysis and swelling of the starch granules in cooked beans. Chung (1996), found that the longer it took beans to cook, the greater the crystallization of the cell walls. In addition, there was variation of cell wall rigidity between navy and pinto beans.

The possibility for genetically improving bean digestibility has been suggested by Murphy (1973). Variability of flatulence response was observed in humans after subjects ate beans from a cross between a reportedly gasless bean variety with a 'normal' bean genotype (Murphy, 1973).

Because of the interest by researchers in improving digestibility in beans and the inclusion of a digestibility improvement component into a breeding program, this study to ascertain variation in starch digestibility was undertaken. The main objective of the research was to measure in vitro starch digestibility in a selected sample of 41 dry bean genetic stocks. A second objective was to ascertain the potential of specific genetic stocks as parents in a breeding program to improve starch digestibility and reduce flatulence.

MATERIALS AND METHODS

Plant Materials

Forty-one genetic stocks (entries) of dry bean (Table 1) from various U.S. and international breeding programs formed the experimental material for this study. The forty-one entries (Table 1) differed from one another for seed color, shape, and size, the commercial class, and yield. The entries had been previously evaluated for adaptation to Michigan bean production areas and several were evaluated for thermal processing traits.

Field Plot Procedure

The forty-one entries were planted in a 6 x 7 rectangular lattice with 3 replications in a Misteguay silty clay soil [fine, ellitic (calcareous), frigid typic Haplaquolls] at the Saginaw Valley Bean and Sugarbeet Research Farm, Saginaw, MI in 1996. Seeds of each entry were precision-drilled with a tractor mounted air planter into 4 row plots that were 5 m long and spaced 0.5 m apart. Within-row spacing was 0.12 m. At the time of planting, a mixed fertilizer consisting of 33.0 kg N· ha^{-1} and 11 kg P· ha^{-1} to which 10 and 2.5% Mn and Zn, respectively, were banded in rows. A pre-plant incorporated application of 4.7 L ·ha^{-1} Eptam (S-ethyl dipropylthiocarbamate) + 2.3 L · ha^{-1} Dual (S-metolachlor) was applied to control weeds. Subsequent hand weeding was practiced as needed. Mature plants of the forty-one entries were harvested and threshed from 8 m of row of each plot. Seeds were cleaned and sized using slotted metal sieves and stored at 23°C until samples were prepared for laboratory analyses.

Table 1. Identification, commercial class, seed coat color, seed weight, and yield of 41 dry bean genotypes.

Identification	Commercial Class	Seed Coat Color	Seed Weight ($g \cdot 100\ seeds^{-1}$)	Yield ($Kg \cdot ha^{-1}$)**
N80242	Small white	White	18	2700
Black Turtle Soup	Tropical black	Black	20	2429
Seafarer	Navy	White	17	2328
Domino	Tropical black	Black	21	3335
Sanilac	Navy	White	21	2739
Tuscola	Tropical black	Black	22	2282
8217-III-24	Undefined	White	19	1648
Jalpataqua	Tropical black	Black	23	3151
Nep-2	Navy	White	18	2200
San Fernando	Tropical black	Black	19	3341
Bunsi	Navy	White	18	2277
ICA Pijao	Tropical black	Black	20	2906
Aurora	Small white	White	14	2322
P766	Undefined	Black	20	2711
Jamapa	Tropical black	Black	20	2953
Protop-P1	Pinto	Brown/mottle	34	1239
FF4-13-MMMM	Tropical black	Black	24	2707
C-20	Navy	White	16	2163
Fleetwood	Navy	White	18	2593
Mexico 12-1	Undefined	Brown	21	2697
Carioca	Pinto	Brown-beige/ mottle	22	3003
Laker	Navy	White	19	2365
Black Magic	Tropical black	Black	19	2956
Swan Valley	Navy	White	18	2741
Midnight	Tropical black	Black	20	2814
BAT 41	Small dark red	Red(opaque)	20	2181
15-R-148	Small dark red	Red (shiny)	20	1470
BAT 1507	Small dark red	Red (shiny)	21	2672
BAC 95	Small dark red	Red (shiny)	21	2807
Harblack (O)	Tropical black	Black (opaque)	19	2786
Harblack (Sh)	Tropical black	Black (shiny)	19	3335
Cumulus	Navy	White	22	2521
N84004	Navy	White	17	2582
C-20 Mutant	Navy	White	19	2118
Jacob's Cattle	Heirloom (specialty)	Red/white spotted	50	1310
Huron	Navy	White	21	2523
Mayflower	Navy	White	19	2809
Albion	Navy	White	19	2555
N87602	Navy	White	17	2720
N78042	Navy	White	21	2699
Huetar	Small dark red	Red (opaque)	21	1733
Mean				2522
LSD (P = 0.05)				754
LSD (P = 0.01)				1009
CV (%)				18.1

*Data on seed class and seed color provided by USDA-ARS Bean Food Quality Genetics program in the Department of Crop and Soil Sciences at Michigan State University.

Indigestible Starch Assay

Indigestible starch was determined on both raw and cooked beans. Raw beans were soaked in water for 12 hours, drained, and lyophilized in a Virtis-Genesis 12EL freeze dryer. Beans were cooked in tin cans following the procedure of Hosfield and Uebersax (1980) and Hosfield *et al.*,(1984). These cooking procedures involved placing beans with a fresh weight equivalent of 100 g total solids into nylon mesh bags and soaking them for 30 min at 25°C followed by a 30 min soak at 91°C. All soaking was done in distilled water to which 0.28 g $CaCl_2$ was added per liter of water to produce a solution containing 100 ppm Ca ion. Soaked beans were cooled and drained for 5 min and placed in number 303 (100 x 75 mm) tin cans. Cans were sealed and processed in a retort without agitation for 45 min at 116°C. When the cans were removed from the retort, they were cooled and stored inverted for a minimum of 2 weeks at room temperature. Cooked beans were removed from the cans, drained and lyophilized in a Virtis-Genesis 12EL freeze dryer.

The freeze-dried raw and cooked bean samples were ground in a mill fitted with a 40-mesh screen for cooked beans and 60-mesh screen for raw beans. Cooked beans ground and sieved through a 40-mesh screen, and raw beans ground and sieved through a 60-mesh screen had comparable particle sizes wich were representative of physiological values for particle size when beans are chewed and swallowed by humans (Heller *et al.*, 1977). After the beans were ground, the milled flour was partitioned into three parts for the following analyses: (1) total protein, (2) total starch, and (3) indigestible starch. The

partitioned flour was placed into capped vials, and then refrigerated until evaluated in the three respective assays. All analyses were performed in duplicate.

Indigestible starch was determined on duplicate raw and cooked samples, using the total dietary fiber (TDF) assay described by Lee *et al.*, (1992) in which digestible macromolecules are removed, followed by the resistant starch determination and the glucose analysis (Johansson *et al.*, 1984).

Indigestible Protein Determination

Kjeldahl nitrogen methodology (AACC method 46-12, 1983) was used to determine the content of protein in each raw and cooked bean sample from the TDF assay. Evaluations were performed on one-half of the TDF assay residue remaining after enzymatic digestions of bean samples from each duplicate. A catalyst tablet and 5 ml of sulfuric acid were added to each digestion tube containing the preweighed TDF assay residue. Tubes were place in a digestion block and heated slowly to 400°C for five hours. When digestion was completed, tubes containing the samples were allowed to cool before the distillation and titration procedures were performed. To calculate the percentage of protein in each bean sample, the percentage of nitrogen was multiplied by 6.25, and the following equation was used:

$$\% \text{ protein in the sample} = \frac{(\text{ml of HCl titrated})\,(\text{Normality of HCl})\,(1.4007)\,(6.25)}{\text{TDFA residue weight (g)}}$$

Statistical Analysis

All data collected were subjected to an analysis of variance (ANOVA) appropriate to a randomized complete-block design with a split-plot arrangement of treatments. Genotypes were the whole-plot and the cooked and raw treatments were sub-plots. The model used for split-plot experiments showing the separate experimental error variances for the whole-plot and subplot is:

$$Y_{ijk} = \mu + \alpha_i + \rho_k + d_{ik} + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

$$i = 1,..a, \quad k = 1,..r, \quad j = 1,..b$$

Where μ is the general mean, α_i is the effect of the $_i$th level of factor A (genotypes), ρ_k is the effect of the $_k$th block (replications), d_{ik} is the whole-plot random error, β_j is the effect of the $_j$th level of factor B (treatments, cooked and raw), $(\alpha\beta)_{ij}$ is the interaction effect between factors A and B, and e_{ijk} is the subplot random error. The ANOVA for the whole-plot error and the sub-plot error from the previous model with fixed effects for factors A and B is presented in Table 2.

Statistical analyses were executed by SAS (SAS Institute, 1988). Statistical analyses were performed on the cooked and raw bean treatments separately. The variables — indigestible residue, indigestible starch, and resistant starch were evaluated. Mean separations were performed using the LSD test criterion with the mean square error term set at the 95% confidence level.

Table 2. Form for the analyses of variance of several nutritional quality factors of forty-one dry bean genotypes and two preparation methods in a split-plot arrangement of treatments.

Source of variation	Degrees of freedom	Sum of Squares	Mean square expectations
Replicates	r-1	$ab \sum (Y_k - Y...)^2 = SS(Rep)$	
Whole-plot (A)	a-1	$rt \sum (Y_{i..} - Y...)^2 = SS(A)$	$\sigma^2_e + b\sigma^2_d + rb\theta^2_A$
Error (Whole-plot)	(r-1) (a-1)	$b \sum (Y_{ik} - Y_{i..} - Y_{..k} + Y...)^2 = SS(E_A)$	$\sigma^2_e + b\sigma^2_d$
Sub-plot (B)	b-1	$ra \sum (Y_{.j.} - Y...)^2 = SS(B)$	$\sigma^2_e + ra\theta^2_B$
Interaction (A*B)	(a-1) (b-1)	$r \sum (Y_{ij} - Y_{i..} - Y_{.j} . + Y...)^2 = SS(AB)$	$\sigma^2_e + r\theta^2_{AB}$
Error (Sub-plot)	a(r-1)(b-1)	$\sum (Y_{ijk} - Y_{i.k} - Y_{.jk} + Y...)^2 = SS(E_B)$	σ^2_e
Total	rab-1		

where $\theta^2_A = 1/(a-1)\sum\alpha^2_I$, $\theta^2_B = 1/(b-1)\sum\beta^2_j$, $\theta^2_{AB} = 1/(a-1)(b-1)\sum(\alpha\beta)^2_{ij}$

RESULTS AND DISCUSSION

Total starch and protein were determined on each of the 41 cooked bean samples. The total starch of cooked beans ranged from 31.6 g·100 g^{-1} (15-R-148) to 49.8 g·100 g^{-1} (N80242) (Appendix, Table 1). Total protein content of cooked beans ranged from 17.2 g · 100 g^{-1} (N87602) to 27.8 g · 100 g^{-1} (15-R-148) (Appendix, Table 1). A paired comparison t-test (P=0.10, data not given) indicated that the total starch of raw beans was not significantly different than the cooked beans (mean 41.5 cooked beans vs 40.2 raw beans) (Appendix, Table 1). The raw beans ranged in total starch from a low of 31.1 g· 100 g^{-1} for 'Jalpataqua' to a high of 46.8 g· 100 g^{-1} for Jacob's Cattle (Appendix, Table 1). When the 41 entries of raw beans were taken as a whole, raw bean protein was 2% higher than the cooked beans (24.3 vs 22.4, respectively) (Appendix, Table 1). This difference was highly significant based on a paired comparison t-test (P=0.0001, data not given). Wassimi *et al.,* (1988) reported a 2% loss in protein, on average, when beans were cooked compared to the raw bean percentages. Raw bean protein ranged from 18.5 for N87602 to 29.2 for 8217-111-24 (Appendix, Table 1). There was significant variability between duplicate laboratory samples for indigestible protein (Appendix, Tables 2, 3, 4, and 5).

Indigestible protein was determined on duplicate samples of each genotype for raw (Appendix, Tables 2 and 3) and cooked (Appendix, Tables 4 and 5) beans. There were large differences between field replications for the indigestible starch of raw and cooked beans. This variability is obviated by the large coefficients of variation (CV)

noted (Appendix, Table 6). The analysis of variance for total dietary fiber in bean flour (Table 3) indicated significant differences between genotypes (whole-plots) and the raw and cooked treatments (sub-plots). There were no significant differences among genotypes for indigestible starch (Table 4), but the differences among treatments, i.e., raw and cooked beans, when the data were averaged over all the genotypes, were highly significant (Table 4). There was an increased quantity of total dietary fiber for cooked beans (22.1 mg · 100 mg flour $^{-1}$) compared to raw beans (19.9 mg · 100 mg flour $^{-1}$) on an experiment mean basis (Table 5). This 2.2 mg · 100 mg flour $^{-1}$ increase in the dietary fiber of cooked beans compared to raw beans was not significant (data not shown).

The mean indigestible starch on a per gram flour basis was 12.4 g for raw beans and 33.9 g for cooked beans (Table 6). When the indigestible starch was considered, on a per 100 mg starch basis, the raw beans averaged 3.1 mg · 100 mg starch^{-1} over the 41 genotypes and 8.2 mg · 100 mg starch^{-1} averaged over the 41 cooked bean genotypes (Table 7). The finding that cooked beans had increased indigestible starch compared to raw beans agrees with observations made by Chung (1996) who found that cooking beans, even for 10 minutes, increased the amount of indigestible starch compared to raw beans. Considering this data, Chung (1996) concluded that the increase in indigestibility that he observed in cooked versus raw beans was due to polymerization of the cell walls in cotyledons. When cell walls polymerize or become crystalline in structure they may encapsulate the starch granules, thus, rendering the starch granules inaccessible to enzymatic digestion. The observation in the present study where cooked beans had more

Table 3. Analysis of variance for the total dietary fiber from raw and cooked bean samples of 41 dry bean genotypes.

Source Variation	DF	SS	MS	F Value	Pr>F
Replicates	1	0.8	0.8	0.77	0.3865
Genotypes (Whole plot, Factor A)	40	500	13	6.57	0.0001
Replicates *x* Genotype (Whole plot error)	40	76	2.0	1.88	0.0234
*Treatment (Sub-plot, Factor B)	1	198	198	196	0.0001
Genotype *x* Treatment (Interaction A*x*B)	40	59	1.5	1.46	0.1160
Error (Sub-plot error)	41	41	1		
Total	163	875			
CV (%)	4.8				

*Treatments were raw and cooked beans

Table 4. Analysis of variance for the indigestible starch from raw and cooked bean samples of 41 dry bean genotypes.

Source Variation	DF	SS	MS	F Value	Pr>F
Replicates	1	0.000874	0.00874	52	0.0001
Genotypes (Whole plot, Factor A)	40	0.001305	0.000033	1.4	0.1365
Replicates *x* Genotype (Whole plot error)	40	0.000920	0.000023	1.4	0.1654
*Treatment (Sub-plot, Factor B)	1	0.19059	0.019059	1127	0.0001
Genotype *x* Treatment (Interaction A*x*B)	40	0.001126	0.000028	1.7	0.0540
Error (Sub-plot error)	41	0.000693	0.000017		
Total	163	0.023978			
CV (%)	17.7				

*Treatments were raw and cooked beans.

Table 5. Means and low, medium, and high groupings for the total dietary fiber of 41dry bean genotypes prepared by two methods, raw and cooked.

Raw			Cooked		
Genotype	Total Dietary Fiber (mg · 100mg flour $^{-1}$)	Group*	Genotype	Total Dietary Fiber (mg · 100mg flour $^{-1}$)	Group*
N78042	15.1	I	N78042	15.7	I
N80242	17.3	I	Nep-2	17.1	I
Mayflower	17.8	I	N80242	19.1	I
Nep-2	18.0	I	Midnight	19.7	I
Midnight	18.1	I	Mayflower	19.9	I
C-20 Mutant	18.3	II	Swan Valley	20.1	II
Jacob's Cattle	18.4	II	Tuscola	20.4	II
Harblack (O)	18.5	II	C-20 Mutant	20.6	II
Swan Valley	18.7	II	BAT 1507	20.8	II
N84004	18.9	II	N87602	21.4	II
Huron	18.9	II	N84004	21.4	II
Harblack (Sh)	18.9	II	Albion	21.4	II
N87602	19.0	II	Harblack (O)	21.7	II
Albion	19.0	II	Laker	21.7	II
Bunsi	19.4	II	C-20 Mutant	21.9	II
Laker	19.5	II	Harblack (Sh)	21.9	II
C-20 Mutant	19.5	II	Cumulus	21.9	II
Domino	19.7	II	Huetar	21.9	II
Huetar	19.7	II	Mexico 12-1	21.9	II
Seafarer	19.7	II	San Fernando	22.0	II
Cumulus	19.8	II	BAC 95	22.3	II
BAT 41	19.8	II	Seafarer	22.4	II
8217-III-24	20.1	II	Jacob's Cattle	22.4	II
Tuscola	20.2	II	BAT 41	22.4	II
San Fernando	20.4	II	8217-III-24	22.5	II
FF4-13-MMMM	20.6	II	Huron	22.5	II
BAC 95	20.8	II	Domino	22.5	II
Fleetwood	20.9	II	Bunsi	22.5	II
Mexico 12-1	21.0	II	Carioca	22.8	II
Carioca	21.0	II	P766	22.9	II
Black Turtle Soup	21.0	II	Aurora	22.9	II
Aurora	21.1	II	Black Turtle Soup	23.2	II
BAT 1507	21.3	II	Jalpataqua	23.4	II
ICA Pijao	21.3	II	ICA Pijao	23.6	II
Sanilac	21.6	II	Sanilac	23.9	II
Jalpataqua	21.7	II	15-R-148	24.2	III
Black Magic	21.8	II	Black Magic	24.6	III
Protop-P1	22.2	III	FF4-13-MMMM	25.3	III
Jamapa	22.3	III	Fleetwood	25.4	III
P766	22.6	III	Protop-P1	26.1	III
15-R-148	23.3	III	Jamapa	26.3	III
Mean	19.9		Mean	22.1	
CV (%)	2.8		CV (%)	3.4	
LSD	1.5		LSD	1.9	

*Group; I = low, II = intermediate, III = high.

Table 6. Means and low, medium, and high groupings for the indigestible starch on a total flour basis of 41 dry bean genotypes prepared by two methods, raw and cooked.

Raw			Cooked		
Genotype	Indigestible Starch (mg · g flour $^{-1}$)	Group*	Genotype	Indigestible Starch (mg · g flour $^{-1}$)	Group*
Protop-P1	7.1	I	Mayflower	27.0	I
Harblack (Sh)	8.0	I	15-R-148	28.0	I
Midnight	8.1	I	FF4-13-MMMM	28.4	II
C-20 Mutant	8.1	I	N87602	29.4	II
N87602	8.5	I	Jamapa	29.6	II
Cumulus	8.5	I	Jalpataqua	30.2	II
Jacob's Cattle	8.8	I	Harblack (Sh)	30.3	II
N78042	9.5	II	Swan Valley	30.8	II
P766	9.7	II	San Fernando	30.8	II
Swan Valley	9.9	II	Black Magic	31.1	II
Seafarer	10.0	II	Aurora	31.3	II
N80242	10.0	II	Tuscola	31.4	II
Fleetwood	10.2	II	Harblack (O)	31.6	II
Jalpataqua	10.2	II	N78042	31.7	II
FF4-13-MMMM	10.5	II	Fleetwood	31.7	II
Harblack (O)	11.2	II	Mexico 12-1	32.4	II
Carioca	11.2	II	Protop-P1	32.4	II
Nep-2	11.3	II	P766	32.8	II
Jamapa	12.2	II	Nep-2	33.0	II
C-20	12.4	II	8217-III-24	33.1	II
Black Magic	12.4	II	BAT 1507	33.3	II
San Fernando	12.8	II	C-20	33.4	II
N84004	12.9	II	Huetar	33.6	II
Aurora	13.1	II	Domino	33.9	II
Black Turtle Soup	13.1	II	ICA Pijao	33.9	II
ICA Pijao	13.1	II	Jacob's Cattle	34.0	II
Huetar	13.1	II	Midnight	34.2	II
Mayflower	13.3	II	BAC 95	34.3	II
Tuscola	13.3	II	Seafarer	34.5	II
Domino	13.6	II	Huron	34.6	II
15-R-148	13.7	II	Bunsi	34.9	II
Albion	13.9	II	Black Turtle Soup	35.3	II
Sanilac	14.0	II	Laker	36.4	II
Laker	14.2	II	Carioca	36.6	II
BAC 95	14.3	II	C-20 Mutant	37.1	II
Bunsi	15.7	II	Albion	37.1	II
Mexico 12-1	16.0	II	N84004	39.5	II
BAT 1507	16.4	III	BAT 41	41.1	II
Huron	19.8	III	Sanilac	41.9	III
BAT 41	20.9	III	N80242	44.9	III
8217-III-24	21.5	III	Cumulus	48.7	III
Mean	12.4		Mean	33.9	
CV (%)	28.7		CV (%)	15.5	
LSD	0.0		LSD	0.0	

*Group; I = low, II = intermediate, III = high.

Table 7. Means and low, medium, and high groupings for the indigestible starch on a starch basis of 41 dry bean genotypes prepared by two methods, raw and cooked.

Raw			Cooked		
Genotype	Indigestible Starch (mg · 100mg starch^{-1})	Group*	Genotype	Indigestible Starch (mg · 100mg starch^{-1})	Group*
Midnight	1.8	I	Mayflower	6.3	I
Harblack (Sh)	1.8	I	Tuscola	6.7	I
N87602	1.9	I	FF4-13-MMMM	6.7	I
Protop-P1	1.9	I	Mexico 12-1	7.0	I
Jacob's Cattle	1.9	II	Swan Valley	7.2	I
Swan Valley	2.2	II	N78042	7.2	I
Cumulus	2.2	II	Jamapa	7.2	I
N78042	2.3	II	Black Magic	7.3	I
C-20 Mutant	2.3	II	San Fernando	7.3	I
P766	2.5	II	N87602	7.3	I
Seafarer	2.5	II	Jalpataqua	7.4	I
FF4-13-MMMM	2.6	II	Harblack (Sh)	7.5	I
N80242	2.6	II	Jacob's Cattle	7.9	II
Nep-2	2.6	II	Fleetwood	7.9	II
Fleetwood	2.6	II	Nep-2	7.9	II
Carioca	2.9	II	C-20	7.9	II
Jamapa	3.0	II	Harblack (O)	7.9	II
San Fernando	3.0	II	ICA Pijao	7.9	II
Tuscola	3.1	II	Midnight	8.0	II
Albion	3.1	II	Carioca	8.1	II
N84004	3.1	II	Protop-P1	8.2	II
C-20	3.1	II	Albion	8.3	II
Black Turtle Soup	3.2	II	Laker	8.3	II
ICA Pijao	3.3	II	BAT 1507	8.3	II
Jalpataqua	3.3	II	Huetar	8.3	II
Harblack (O)	3.3	II	Seafarer	8.4	II
Huetar	3.4	II	Aurora	8.4	II
BAC 95	3.4	II	Bunsi	8.4	II
Aurora	3.4	II	Domino	8.4	II
Domino	3.4	II	BAC 45	8.5	II
Sanilac	3.5	II	Sanilac	8.5	II
Mayflower	3.5	II	8217-III-24	8.7	II
15-R-148	3.5	II	C-20 Mutant	8.7	II
Black Magic	3.5	II	15-R-148	8.9	II
Laker	3.7	II	Huron	8.9	II
Bunsi	3.7	II	N80242	9.0	II
Mexico 12-1	3.9	II	Black Turtle Soup	9.4	II
Huron	4.4	II	P766	9.8	II
BAT 1507	5.2	III	BAT 41	10.1	III
BAT 41	5.4	III	N84004	10.3	III
8217-III-24	5.6	III	Cumulus	11.9	III
Mean	3.1		Mean	8.2	
CV (%)	28.6		CV (%)	15.0	
LSD	1.8		LSD	2.5	

*Group; I = low, II = intermediate, III = high.

indigestible starch than raw beans may be due to the cell wall polymerization phenomenon described by Chung (1996). Cooking beans also increased the percentage of indigestible protein compared to that found in raw beans (Table 8).

There was considerable variation associated with the assays for indigestible starch and indigestible protein (Tables 6, 7, and 8). The coefficients of variability for indigestible starch of the 41 genotypes were 28.7 % (Table 6) and 28.6% (Table 7) for raw beans and 15.5% (Table 6) and 15.0% (Table 7) for cooked beans on a per mg of flour and per 100 mg of starch basis, respectively. There was large variability for indigestible protein of raw and cooked beans denoted by the coefficients of variability. The coefficients of variability were 34.9% and 27.5% for the raw and cooked samples, respectively (Table 8).

The variability noted among data for indigestible starch (Tables 6, 7), and protein (Table 8) was most likely due to analytical error. The methodology to determine indigestible components in raw and cooked bean flour involved (1) grinding beans to a small and homogeneous particle size, (2) determining the indigestible residue, and (3) determining the undigested starch and protein that is in the indigestible residue. Since the determination of indigestible starch and protein required scraping small samples of residue from the sintered glass crucibles, pipetting small quantities of solution, and reading glucose formation from 40 ml of solution; small analytical errors could be magnified by the various multiplication factors used in calculating data, thus, leading to large errors in the final values calculated.

The four genotypes with the highest quantities of total dietary fiber in the raw

Table 8. Means and low, medium, and high groupings for the indigestible protein as a percent of the total protein of 41 dry bean genotypes prepared by two methods, raw and cooked.

Indigestible Protein (as a percentage of the total seed protein)					
	Raw			Cooked	
Genotype	(%)	Group*	Genotype	(%)	Group*
Laker	6.4	I	Swan Valley	10.2	I
Sanilac	9.1	I	Laker	12.0	I
8217-III-24	9.2	I	N78042	12.2	I
Albion	9.9	I	Fleetwood	12.6	I
Swan Valley	10.1	I	Huron	12.9	I
C-20 Mutant	10.5	I	Sanilac	12.9	I
Tuscola	10.7	I	Tuscola	13.5	I
N84004	11.0	II	N84004	14.0	I
N87602	11.1	II	Jacob's Cattle	14.1	I
N80242	11.1	II	N80242	14.1	I
N78042	11.8	II	BAC 95	14.4	I
Huron	12.1	II	C-20	15.8	I
Domino	12.1	II	Carioca	16.3	II
Seafarer	12.5	II	Bunsi	17.3	II
San Fernando	12.6	II	Aurora	17.5	II
Harblack (O)	12.6	II	Albion	17.6	II
FF4-13-MMMM	12.8	II	8217-III-24	17.8	II
Jamapa	13.0	II	Mayflower	18.0	II
Bunsi	13.2	II	15-R-148	18.3	II
Mayflower	13.8	II	BAT 41	18.4	II
Jalpataqua	13.9	II	San Fernando	18.6	II
Black Turtle Soup	14.0	II	Mexico 12-1	18.7	II
BAC 95	14.0	II	ICA Pijao	18.8	II
Protop-P1	14.1	II	Nep-2	19.4	II
Fleetwood	14.4	II	Black Magic	19.8	II
C-20	14.6	II	Black Turtle Soup	19.9	II
BAT 41	14.8	II	Midnight	19.9	II
Mexico 12-1	14.9	II	Jalpataqua	20.5	II
Aurora	15.0	III	Cumulus	20.7	II
Carioca	15.4	III	FF4-13-MMMM	20.7	II
P766	15.5	III	Harblack (O)	20.9	II
ICA Pijao	16.5	III	C-20 Mutant	21.8	II
Nep-2	16.6	III	N87602	21.8	II
Harblack (Sh)	17.0	III	Protop-P1	22.6	III
Huetar	17.5	III	Huetar	22.6	III
BAT 1507	18.0	III	Domino	23.1	III
15-R-148	18.5	III	Harblack (Sh)	23.3	III
Cumulus	18.9	III	BAT 1507	23.3	III
Black Magic	19.3	III	P766	25.5	III
Jacob's Cattle	20.0	III	Seafarer	25.6	III
Midnight	20.8	III	Jamapa	26.6	III
Mean	13.7		Mean	17.9	
CV (%)	34.9		CV (%)	27.5	
LSD	1.8		LSD	2.5	

*Group; I = low, II = intermediate, III = high.

bean treatment were Protop-P1, 'Jamapa,' P766, and 15-R-148 (Table 5). These four lines made up the high group of lines for total dietary fiber and ranged from 22.2 to 23.3 mg· 100 mg flour $^{-1}$ (Table 5). The raw bean entries, N78042, N80242, 'Mayflower,' Nep-2, and 'Midnight' formed the group with the lowest values for dietary fiber (Group I) (Table 5). The dietary fiber values for this group ranged from 15.1 to 18.1 mg·100 mg^{-1} of flour. The remaining 32 genotypes exhibited intermediate dietary fiber values and ranged from 18.3 to 21.8 mg of dietary fiber · 100 mg^{-1} of flour (Table 5).

Although the ranges of dietary fiber values for cooked beans (Table 5) was higher than the raw beans—15.7 to 26.3 (cooked beans) versus 15.1 to 23.3 (raw beans) mg of dietary fiber · 100 mg^{-1} of flour, respectively— many of the same genotypes fell into the same respective groupings noted for the raw beans. For example, 15-R-148, Protop-P1, and 'Jamapa' were in the high group (Group III) for total dietary fiber of cooked beans and also in the high group for the total dietary fiber of raw beans (Table 5). The five genotypes that made-up the low group (Group I) of entries for total dietary fiber were the same for both raw and cooked beans.

The genotypes Nep-2 and 'San Fernando' were expected to be close in mean values for total dietary fiber but fell into different groups —low (Nep-2) and intermediate (San Fernando), respectively— in the cooked treatment (Table 3). Nep-2, a white seeded bean, is a EMS mutant of 'San Fernando,' a black seeded bean (Moh, 1971), and presumably differs from San Fernando only by the P allele the, "ground gene" of Kooiman, (1931), which is necessary for the plant to produce color in the seed coat. The

difference in dietary fiber between 'San Fernando' and Nep-2 may be due to flavonoid compounds present in black beans (Beninger *et al.*, 2000) but not in white beans that can complex with seed components, namely starch and protein, thus rendering these macromolecules indigestible. An increased indigestible starch and/or protein in the seed would be reflected in a higher quantity of total dietary fiber.

'Jacob's Cattle,' that was evaluated by Murphy, (1973), and reported as a probable gasless variety, had a higher amount of dietary fiber when seeds were cooked than in the raw seeds. Other genotypes had lower values for dietary fiber of raw and cooked beans than 'Jacob's Cattle' (Table 5).

Mean values of indigestible starch on a per gram flour basis for the individual genotypes for raw beans ranged from 7.1 to 21.5 mg (Table 6) and 1.8 to 5.6 mg on a 100 mg of starch basis (Table 7). For raw bean flour, the genotype 8217-III-24 had the highest amount of indigestible starch on both a per gram flour and per 100 mg of starch basis (Tables 6 and 7, respectively). Other genotypes with high quantities of indigestible starch on a per gram flour basis in the raw bean were BAT 41 and 'Huron' with 20.9 and 19.8 mg $\cdot$ g^{-1} flour, respectively (Table 6). In the raw bean, Protop-P1 had the lowest amount of indigestible starch on a per gram flour basis (Table 6), but 'Midnight' had the least amount of indigestible starch on a per 100 mg of starch basis (Table 7). In the raw bean, low quantities of indigestible starch on a per gram of flour (Table 6) were also associated with the genotypes 'Harblack (Sh)', 'Midnight,' C-20 Mutant, N87602, 'Cumulus', and 'Jacob's Cattle.' The range of values for the genotypes in the low

indigestible starch on a per gram flour basis was 7.1 to 8.8 mg · g^{-1} of flour (Table 6).

For the cooked beans, means of the individual genotypes for indigestible starch ranged from 27.0 mg to 48.7 mg on a per g of flour basis (Table 6) and 6.3 to 11.9 mg on a per 100 mg of starch basis (Table 7). 'Cumulus' had the highest level of indigestible starch on both a per g of flour and 100 mg starch basis (Tables 6 and 7). In the cooked bean group, 'Mayflower' had the lowest amount of indigestible starch on both a per g of flour and 100 mg of starch basis. Spearman's rank correlation (r_s statistic) (Appendix, Tables 7, 8 and 9) computed between the ranks of individual genotypes for indigestible starch on a per g of flour basis and on a per mg starch basis were high value and highly significant for both raw (r_s=0.9) and cooked (r_s=0.7) beans, indicating that the genotypes ranked similarly for indigestible starch on a per g of flour basis and per 100 mg starch basis.

Comparison of the rankings of means for total dietary fiber (Table 5) and indigestible starch on a per g flour basis when beans were cooked (Table 6), showed that 15-R-148 had a high quantity of total dietary fiber (24.2 mg · 100 mg flour $^{-1}$) but a low quantity of indigestible starch on a per g flour basis (28.0 mg · g flour $^{-1}$). Although the indigestible starch quantity of 15-R-148 was comparatively low on a per g flour basis, the indigestible starch of this entry on a per 100 mg starch basis tended to be high (8.9 mg · 100 mg^{-1}) (Table 7) compared to the other entries. 15-R-148 had the lowest amount of total starch 31.6 g · 100 mg^{-1} of the 41 entries evaluated (Appendix, Table 1). Also among the cooked bean samples, 'Sanilac' had the highest amount of total dietary

fiber of the intermediate ranked group for this trait (Table 5), but was in the group that ranked high for the amount of indigestible starch on a per g flour basis (Table 6). There does not appear to be a strong association between the quantity of total dietary fiber and indigestible starch when all 41 entries are considered together. Single degree of freedom analyses for total dietary fiber and indigestible starch on a per g flour basis showed that 12 of the 41 entries evaluated had non-significant mean squares for the total dietary fiber trait but all the entries had significant mean square for the indigestible starch on a per mg flour basis (Table 9).

'Mayflower' contrasted with 15-R-148 for quantities of total dietary fiber and indigestible starch on both a per g flour and 100 mg starch basis of cooked beans. 'Mayflower' had a low quantity of indigestible starch on a per g flour basis (27.0 mg · g^{-1}) (Table 6) and per mg starch basis (6.3 mg · 100 mg^{-1}) (Table 7). The total starch content of cooked 'Mayflower' bean was comparatively high (42.9 g · 100 g^{-1}) among the 41 entries and exceeded the mean of the entries by 1.4 g · 100 g^{-1} (Appendix, Table 1). Other entries such as 'Tuscola,' 'Swan Valley,' and N78042 fell into the low or low side of the intermediate group for total dietary fiber (Table 5) and had low amounts of indigestible starch on a per 100 mg starch basis (Table 7), but their quantities of indigestible starch on a per g flour basis (Table 6) were intermediate between the high and low groupings.

Means for indigestible protein of cooked beans (Table 8) ranged from 10.2 % ('Swan Valley') to 26.6% ('Jamapa') and 6.4% ('Laker') to 20.8% ('Midnight') for raw beans (Table 8). Cooked bean genotypes, 'Seafarer' (25.6%), P766 (25.5%), 'Domino'

Table 9. Mean squares for the variables total dietary fiber and indigestible starch of the treatment effect in each genotype.

Identification	df	Total Dietary Fiber (mg/100 mg flour)	Indigestible Starch (mg/g flour)
N80242	1	1.8162*	0.001224**
Black Turtle Soup	1	2.2943*	0.000494**
Seafarer	1	3.4172*	0.000601**
Domino	1	4.0384**	0.000409**
Sanilac	1	2.4315*	0.000776**
Tuscola	1	0.0132 NS	0.000326**
8217-III-24	1	2.7114*	0.000135**
Jalpataqua	1	1.3268 NS	0.000398**
Nep-2	1	0.4628 NS	0.000472**
San Fernando	1	1.3719 NS	0.000328**
Bunsi	1	5.0711**	0.000369**
ICA Pijao	1	2.5106*	0.000435**
Aurora	1	1.7715*	0.000331**
P766	1	0.0344 NS	0.000534**
Jamapa	1	7.2983**	0.000304**
Protop-P1	1	6.8787**	0.000641**
FF4-13-MMMM	1	10.5034**	0.000319**
C-20	1	2.7620*	0.000442**
Fleetwood	1	9.1150**	0.000465**
Mexico 12-1	1	0.5120 NS	0.000271**
Carioca	1	1.5640*	0.000646**
Laker	1	2.4747*	0.000490**
Black Magic	1	3.5054**	0.000349**
Swan Valley	1	1.0686 NS	0.000439**
Midnight	1	1.4702 NS	0.000684**
BAT 41	1	3.4966*	0.000407**
15-R-148	1	0.4173 NS	0.000204**
BAT 1507	1	0.0936 NS	0.000287**
BAC 95	1	1.0468 NS	0.000397**
Harblack (O)	1	4.9608**	0.000419**
Harblack (Sh)	1	4.3942**	0.000499**
Cumulus	1	2.3328*	0.001614**
N84004	1	3.2146*	0.000712**
C-20 Mutant	1	3.2146*	0.000712**
Jacob's Cattle	1	8.2723**	0.000635**
Huron	1	6.6973**	0.000218**
Mayflower	1	2.3709*	0.000188**
Albion	1	2.9088*	0.000540**
N87602	1	2.8614*	0.000438**
N78042	1	0.2601 NS	0.000492**
Huetar	1	2.3973*	0.000418**

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively; NS = Non significant

(23.1%), BAT 1507 (23.3%), and 'Harblack (Sh)' (23.3%) had a high quantity of indigestible protein. 'Swan Valley' (10.2%), 'Laker' (12.0%), and N78042 (12.2%) had low quantities of indigestible protein (Table 8). In the raw bean, genotypes 'Midnight' (20.8%), 'Jacob's Cattle' (20.0%), and 15-R-148 (18.5%) showed high quantities of indigestible protein, and 'Laker' (6.4%), 'Mayflower' (13.8%), and N87602 (11.1%) had a low percentage of indigestible protein (Table 8). Samples of cooked beans of 'Jamapa' and N78042 showed high and low mean values, respectively for both total dietary fiber (Table 5) and indigestible protein (Table 8). The raw samples of genotypes 15-R-148 and 'Mayflower' had high and low or intermediate contents, respectively of total dietary fiber (Table 5) and indigestible protein (Table 8).

The mean square for duplicates for indigestible protein was significant (α=0.05) indicating a lack of consistency among the two laboratory analyses for each genotype. The lack of consistency between duplicate samples probably contributed to the large coefficient of variability (Table 8).

Breeding implications.

A loss of nutrients occurs when a food component is not digested and absorbed in the small intestine. Moreover, undigested food provides a substrate for microbial digestion in the colon with accompanying gastrointestinal discomfort including flatus production. Flatulence occurring after consuming a meal of beans is considered by many as the most important factor limiting the consumption of dry bean. Hence, the

improvement of digestibility in dry bean would not only improve the bioavailability of starch and protein but also reduce flatulence from eating beans and enhance consumer acceptance of this important crop.

Significant variability among genetic stocks for indigestible components of the seed –total dietary fiber, starch, and protein– found in this study indicate that these traits can be altered by selection. Improved digestibility of macromolecules and a reduction in flatus potential could probably be achieved by selecting in breeding populations those recombinants with low quantities of indigestible components. Generally, the breeding objectives of a program dictate which traits are selected and procedures used. In this regard, the bean breeder interested in improving the food value of the crop is faced with several challenges. The questions proposed for the present study include: should the breeder concentrate selection efforts to reduce flatulence, reduce indigestible starch, —thus improving the bioavailability of this nutrient — or improve the total dietary fiber component? Total dietary fiber consists of the indigestible residue after bean flour is treated with digestive enzymes and is composed mainly of cell wall components (cellulose and hemicellulose), lignin, undigested starch, protein, sugars, and ash. By reducing the indigestible residue through selection, the breeder is effectively reducing total dietary fiber. Since dietary fiber is an important component in health improvement strategies to reduce serum cholesterol and the incidence of some types of cancer, selection for a reduced content of indigestible residue (total dietary fiber) in bean seeds may have a negative impact on improving dry bean for some factors contributing to

human health. However, selection for a low dietary fiber content would be in agreement with a breeder's desire to improve digestibility and lower flatulence. In other words, one of the characteristics of beans that promote their utility as a healthy food — a good source of fiber — is a characteristic that limits bean consumption because of the potential for gastrointestinal stress including flatulence. Faced with this dilemma, the breeder might proceed with a strategy that permits one to increase the quantity of total dietary fiber (indigestible residue) and lower the quantity of indigestible starch. Although the correlation between the total dietary fiber and indigestible starch was not determined in this study, there does not appear to be a strong association between these two traits (Tables 5, 6, and 7). For example, 'Jamapa' had the highest quantity of total dietary fiber in cooked beans (26.3%) (Table 5) but had a relatively low quantity (Group II) of indigestible starch on a per g flour basis (Table 6) compared to the other genotypes and ranked 37th highest for this trait. Other examples of the lack of a strong association between a line's total dietary fiber and indigestible starch are provided in Tables 5 and 6. The development of a selection index along with selection either upward or downward to some ideal value for each of the traits may be a method to increase total dietary fiber and decrease indigestible starch in beans through selection and breeding.

Seed characteristics that could be involved in breeding for improved carbohydrate digestibility in beans are total starch, indigestible residue (or dietary fiber), and indigestible starch. Digestibility determinations can be made on a per gram of flour used for laboratory evaluations or on a per 100 mg of starch determined from total starch values of beans. After the evaluation of these parameters, the breeder would decide which

one to use in the selection program. A useful parameter for the breeder to consider to improve the digestibility of beans through selection is the indigestible starch on a per 100 mg of starch basis. A bean genotype with low indigestible starch on a per 100 mg of starch, although containing high indigestible starch on a per g of flour, would be a good parent to include in a plant breeding program. Determination of total dietary fiber could be considered the most cost efficient evaluation since it is obtained directly from the enzyme digestions during the assay. The total dietary fiber is the indigestible residue remaining after the flour is digested with enzymes and, thus, contains the undigested macromolecules present in the flour sample.

The results of this study point out the need to determine both the total seed starch (e.g. appendix Table 1) and the indigestible starch on a per g of flour basis (e.g. Table 6). In a breeding program the selection of a segregant on the basis of high total starch and low indigestible starch (per g of flour) would be equivalent to selecting a segregant with low total starch and high indigestible starch (per g of flour).

In Phaseolus vulgaris evidence indicates two major centers of domestication —in Mesoamerica and in the Andes— which led to two groups of cultivars with contrasting agronomic characteristics (Gepts and Debouck, 1991). These two gene pools are distinguished by their unique electrophoretic pattern of phaseolin —a reserved seed protein (Gepts and Bliss, 1986; Gepts *et al.*, 1986; and Gepts, 1988). Cultivars with "S" type phaseolin generally have comparatively small seeds and are classified in the Mesoamerican center of domestication; cultivars with "T" type phaseolin generally have

large seeds (e.g. Kidney beans) and are classified in the Andean center of domestication. In this study all the bean genetic stocks used except Jacob's Cattle represented the Mesoamerican center of domestication. Jacob's Cattle total dietary fiber and contents of indigestible starch were unremarkable for cooked beans; however, in a separate study (Ospina, unpublished, 1999) 'Montcalm' dark red kidney bean — a genotype representing the Andean center of domestication — had an indigestible starch value for cooked beans of 22.7 mg · g flour $^{-1}$ (data not shown). This value was 4 mg lower than 'Mayflower' a Mesoamerican cultivar evaluated in the current study with the lowest value among genetic stocks for cooked bean indigestible starch (on a per g flour basis) (Table 6). The low content of indigestible starch in 'Montcalm' suggests that there could be a possible difference between the Andean and Mesoamerican bean genotypes for total dietary fiber and starch digestibility. Future research could determine if there is a relationship between genetic origin of a bean cultivar and its content of indigestible starch. Although there is no published data on starch indigestibility in dry beans and the minimum and maximum values for this trait are currently unknown, results from the present study are useful as a point of reference for bean breeders. The indigestible starch content of 'Montcalm' indicates that it would be wise to evaluate germplasm with more genetic diversity.

GENERAL CONCLUSIONS

In the determination of indigestible starch in dry beans, comparable particle size of the cooked and raw bean flour is important to obtain accurate results. To obtain comminuted cooked and raw bean flour with similar particle sizes, cooked beans should be freeze dried and ground through a 40-mesh screen, and raw beans should be soaked, freeze dried, and ground through a 60-mesh screen. The grinding of raw beans without the soaking process caused frequent screen breakage when ground through a 60-mesh screen, thus, rendering flour from raw, non-soaked beans unacceptable because of wide variability of particle size. Humidification of bean seed was time consuming and made this process impractical. The measurement of the particle size distribution of bean flour by using several different sizes of mesh screens was a useful way to compare the particle size of bean flour regardless of treatment. Differences among genotypes for indigestible starch in raw and cooked beans could be detected using enzymatic methodologies once raw and cooked bean particle sizes were comparable.

Significant differences were detected between the experiment means for indigestible starch on both a per gram flour and per 100 mg starch basis of raw and cooked seeds of the 41 genotypes evaluated in the present study. However, no differences were detected between the experiment means for raw and cooked beans for total dietary fiber. Several genotypes had similar values for dietary fiber regardless of whether the flour samples came from cooked or raw beans. This finding suggests that cooking of beans does not always increase the amount of indigestible residue in beans.

Also, it could be inferred that bean genotypes with high dietary fiber content and low indigestible starch have high amounts of indigestible protein and ash. Evaluation of indigestible protein in the 41 bean genotypes showed that cooked samples had higher values than raw samples, except for Jacob's Cattle. Beans are known to lose protein during cooking and the amount lost may be as high as 2%.

Results obtained in this study suggest that there could be a possible difference in content of indigestible starch between Andean and Mesoamerican bean genotypes. Further research is necessary to determine if there is a relationship between genetic origin of the bean genotype and its content of indigestible starch.

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APPENDIX

Table 1. Means for total starch and total starch and total protein on a total flour basis for 41 dry bean genotypes prepared by two methods, raw and cooked.

	Total Starch ($g \cdot 100g^{-1}$)		Total Protein ($g \cdot 100g^{-1}$)	
Genotype	Cooked	Raw	Cooked	Raw
N80242	49.8	38.6	20.1	24.6
Black Turtle Soup	37.6	41.2	20.8	22.5
Seafarer	41.2	40.1	20.8	22.1
Domino	40.3	39.9	24.2	26.1
Sanilac	49.3	40.6	21.0	23.7
Tuscola	46.5	43.5	24.4	25.4
8217-III-24	38.1	38.3	22.4	29.2
Jalpataqua	40.7	31.1	22.9	25.5
Nep-2	41.7	43.3	23.4	25.1
San Fernando	42.4	41.9	23.7	25.7
Bunsi	41.6	42.3	23.0	27.3
ICA Pijao	42.8	40.4	22.7	24.9
Aurora	37.3	38.3	24.0	26.4
P766	33.5	39.0	22.6	27.7
Jamapa	41.1	41.3	20.6	24.8
Protop-P1	39.5	37.8	26.0	29.1
FF4-13-MMMM	42.0	40.9	21.2	22.4
C-20	42.2	39.4	21.8	25.0
Fleetwood	40.3	38.4	20.9	23.4
Mexico 12-1	46.0	41.1	22.0	20.5
Carioca	45.2	38.8	21.8	22.2
Laker	43.8	39.0	22.9	23.8
Black Magic	42.8	35.4	21.8	24.4
Swan Valley	43.0	44.7	24.0	24.9
Midnight	43.0	46.2	21.7	23.3
BAT 41	40.9	38.8	24.6	25.1
15-R-148	31.6	39.2	27.8	28.5
BAT 1507	40.1	31.3	23.4	25.1
BAC 95	40.4	42.2	23.0	22.0
Harblack (O)	39.9	33.6	20.4	22.8
Harblack (Sh)	40.4	44.1	21.1	21.8
Cumulus	38.5	41.3	22.1	24.4
N84004	38.5	41.3	24.5	24.8
C-20 Mutant	42.4	35.0	20.1	22.6
Jacob's Cattle	43.3	46.8	21.7	23.3
Huron	38.8	44.8	24.2	21.8
Mayflower	42.9	38.3	23.7	25.3
Albion	44.9	44.8	19.8	21.1
N87602	40.0	45.5	17.2	18.5
N78042	44.0	41.3	21.1	22.9
Huetar	40.3	39.1	21.7	25.1
Experiment mean	41.5	40.2	22.4	24.3
CV (%)	8.2	8.9	8.4	9.3

Table 2. Indigestible protein in raw beans in duplicate 'A'.

Identification	Protein in residue (g)	Residue from TDF (g)	Indigestible protein in residue (g)	Total protein flour (g)	Initial wt sample for TDF (g)	Total protein sample (g)	Indigestible protein (g)
N80242	0.316	0.1518	0.02064	0.24545	1.0008	0.224565	0.0919
Black Turtle Soup	0.173	0.1968	0.03405	0.22521	1.0010	0.225435	0.1510
Seafarer	0.152	0.1955	0.02972	0.02972	1.0012	0.215809	0.1377
Domino	0.198	0.2057	0.04073	0.26106	1.0013	0.261399	0.1558
Sanilac	0.129	0.2111	0.02723	0.23729	1.0007	0.237456	0.1147
Tuscola	0.157	0.1997	0.03135	0.25376	1.0001	0.253785	0.1235
8217-III-24	0.185	0.1926	0.03563	0.03563	1.0016	0.292047	0.1220
Jalpataqua	0.162	0.2180	0.03532	0.25476	1.0007	0.254938	0.1385
Nep-2	0.245	0.1859	0.04555	0.25111	1.0013	0.251436	0.1811
San Fernando	0.141	0.1926	0.02716	0.25635	1.0006	0.256504	0.1059
Bunsi	0.210	0.1886	0.03961	0.27237	1.0004	0.272479	0.1454
ICA Pijao	0.205	0.1935	0.03967	0.24896	1.0009	0.249184	0.1592
Aurora	0.255	0.2084	0.05314	0.26427	1.0015	0.264666	0.2008
P766	0.192	0.2396	0.46000	0.27695	1.0017	0.277421	0.1658
Jamapa	0.148	0.2219	0.24834	0.24834	1.0008	0.248539	0.1321
Protop-P1	0.197	0.2245	0.04423	0.29085	1.0016	0.291315	0.1518
FF4-13-MMMM	0.137	0.1972	0.02642	0.22355	1.0004	0.223639	0.1181
C-20	0.175	0.1970	0.03448	0.24967	1.0018	0.250119	0.1379
Fleetwood	0.197	0.2089	0.04115	0.23403	1.0011	0.234287	0.1756
Mexico 12-1	0.181	0.2067	0.03742	0.20458	1.0015	0.204887	0.1826
Carioca	0.182	0.2201	0.04006	0.22144	1.0011	0.221684	0.1807
Laker	0.069	0.2111	0.01457	0.23813	1.0012	0.238416	0.0611
Black Magic	0.292	0.2236	0.06529	0.24420	1.0002	0.244249	0.2673
Swan Valley	0.126	0.2048	0.02580	0.24944	1.0016	0.249839	0.1033
Midnight	0.322	0.2082	0.06704	0.23259	1.0018	0.233009	0.2877
BAT 41	0.159	0.2117	0.03366	0.25071	1.0013	0.251036	0.1341
15-R-148	0.207	0.2593	0.05368	0.28461	1.0013	0.284980	0.1884
BAT 1507	0.212	0.2071	0.04391	0.25082	1.0011	0.251096	0.1749
BAC 95	0.103	0.2096	0.02159	0.21960	1.0009	0.219798	0.0982
Harblack (O)	0.132	0.1868	0.02466	0.22753	1.0006	0.227667	0.1083
Harblack (Sh)	0.200	0.1931	0.03862	0.21794	1.0003	0.218005	0.1772
Cumulus	0.270	0.2006	0.05416	0.24412	1.0014	0.244462	0.2215
N84004	0.110	0.1799	0.01979	0.24805	1.0013	0.248372	0.0797
C-20 Mutant	0.130	0.1807	0.02349	0.22591	1.0011	0.226159	0.1039
Jacob's Cattle	0.288	0.2049	0.05901	0.23296	1.0004	0.233053	0.2532
Huron	0.120	0.2011	0.02413	0.21803	1.0009	0.218226	0.1106
Mayflower	0.190	0.1799	0.03418	0.25212	1.0019	0.252599	0.1353
Albion	0.082	0.1956	0.01604	0.21124	1.0013	0.211515	0.0758
N87602	0.106	0.1933	0.02049	0.18507	1.0007	0.185200	0.1106
N78042	0.197	0.1549	0.03052	0.22911	1.0018	0.229522	0.1330
Huetar	0.303	0.1914	0.05799	0.25025	1.0010	0.250500	0.2315

Table 3. Indigestible protein in raw beans in duplicate 'B'.

Identification	Protein in residue (g)	Residue from TDF (g)	Indigestible protein in residue (g)	Total protein flour (g)	Initial wt sample for TDF (g)	Total protein sample (g)	Indigestible protein (g)
N80242	0.185	0.1716	0.031746	0.24545	1.0014	0.245794	0.1292
Black Turtle Soup	0.145	0.1995	0.028928	0.22521	1.0014	0.225525	0.1283
Seafarer	0.126	0.1926	0.024268	0.21555	1.0004	0.215636	0.1125
Domino	0.113	0.1978	0.022351	0.26106	1.0017	0.261504	0.0855
Sanilac	0.074	0.2163	0.016006	0.23729	1.0012	0.237575	0.0674
Tuscola	0.115	0.1997	0.022966	0.25376	1.0001	0.253785	0.0905
8217-III-24	0.093	0.1961	0.018238	0.29158	1.0003	0.291667	0.0625
Jalpataqua	0.161	0.2114	0.035645	0.25476	1.0014	0.255117	0.1397
Nep-2	0.245	0.1541	0.037755	0.25111	1.0016	0.251512	0.1501
San Fernando	0.214	0.1757	0.037599	0.25635	1.0020	0.256863	0.1464
Bunsi	0.168	0.1933	0.032474	0.27237	1.0007	0.272561	0.1191
ICA Pijao	0.203	0.2110	0.042833	0.24896	1.0018	0.249408	0.1717
Aurora	0.128	0.2028	0.025958	0.26427	1.0005	0.264402	0.0982
P766	0.179	0.2251	0.040293	0.27695	1.0011	0.277255	0.1453
Jamapa	0.148	0.2160	0.031968	0.24834	1.0005	0.248464	0.1287
Protop-P1	0.197	0.1935	0.038119	0.29085	1.0012	0.291199	0.1309
FF4-13-MMMM	0.149	0.2072	0.030873	0.22355	1.0011	0.223796	0.1380
C-20	0.201	0.1923	0.038652	0.24967	1.0018	0.250119	0.1545
Fleetwood	0.127	0.2086	0.026492	0.23403	1.0009	0.234241	0.1131
Mexico 12-1	0.121	0.1952	0.023619	0.20458	1.0018	0.204948	0.1152
Carioca	0.138	0.2049	0.028276	0.22144	1.0010	0.221661	0.1276
Laker	0.082	0.1961	0.016080	0.23813	1.0013	0.238440	0.0674
Black Magic	0.144	0.2008	0.028915	0.24420	1.0017	0.244615	0.1182
Swan Valley	0.127	0.1939	0.024625	0.24944	1.0017	0.249864	0.0986
Midnight	0.183	0.1641	0.030030	0.23259	1.0007	0.232753	0.1290
BAT 41	0.217	0.1875	0.040688	0.25071	1.0017	0.251136	0.1620
15-R-148	0.220	0.2356	0.051832	0.28461	1.0013	0.284979	0.1819
BAT 1507	0.230	0.2012	0.046276	0.25082	1.0019	0.251297	0.1841
BAC 95	0.198	0.2024	0.040075	0.21960	1.0007	0.219754	0.1824
Harblack (O)	0.223	0.1790	0.039917	0.22753	1.0015	0.227891	0.1431
Harblack (Sh)	0.200	0.1780	0.035600	0.21794	1.0009	0.218136	0.1632
Cumulus	0.191	0.2004	0.038276	0.24412	1.0020	0.244608	0.1565
N84004	0.180	0.1897	0.034146	0.24805	1.0006	0.248199	0.1376
C-20 Mutant	0.130	0.1862	0.024206	0.22591	1.0010	0.226136	0.1070
Jacob's Cattle	0.183	0.1872	0.034258	0.23296	1.0019	0.233403	0.1468
Huron	0.154	0.1857	0.028598	0.21803	1.0016	0.218379	0.1310
Mayflower	0.190	0.1876	0.035644	0.25212	1.0013	0.252448	0.1412
Albion	0.137	0.1881	0.025770	0.22124	1.0013	0.211515	0.1218
N87602	0.106	0.1946	0.020628	0.18507	1.0015	0.185348	0.1113
N78042	0.170	0.1399	0.023783	0.22911	1.0014	0.229431	0.1037
Huetar	0.156	0.1897	0.029593	0.25025	1.0011	0.250525	0.1181

Table 4. Indigestible protein in cooked beans in duplicate 'A'.

Identification	Protein in residue (g)	Residue from TDF (g)	Indigestible protein in residue (g)	Total protein flour (g)	Initial wt sample for TDF (g)	Total protein sample (g)	Indigestible protein (g)
N80242	0.175	0.1842	0.03224	0.20148	1.0005	0.201581	0.1599
Black Turtle Soup	0.195	0.2338	0.04559	0.20758	1.0010	0.207788	0.2194
Seafarer	0.217	0.2302	0.04995	0.20803	1.0001	0.208051	0.2401
Domino	0.228	0.2358	0.05376	0.24164	1.0012	0.241930	0.2222
Sanilac	0.148	0.2412	0.03570	0.21039	1.0013	0.210664	0.1694
Tuscola	0.165	0.2341	0.03863	0.24442	1.0011	0.244669	0.1579
8217-III-24	0.176	0.2204	0.03879	0.22445	1.0018	0.224854	0.1725
Jalpataqua	0.172	0.2393	0.04116	0.22891	1.0012	0.229185	0.1796
Nep-2	0.35	0.1579	0.05527	0.23408	1.0010	0.234314	0.2359
San Fernando	0.182	0.2347	0.04272	0.23699	1.0015	0.237345	0.1799
Bunsi	0.192	0.2270	0.04358	0.23019	1.0007	0.230351	0.1892
ICA Pijao	0.134	0.2301	0.03083	0.22689	1.0009	0.227094	0.1357
Aurora	0.191	0.2158	0.04122	0.23961	1.0010	0.239850	0.1718
P766	0.319	0.2556	0.08154	0.22626	1.0014	0.226577	0.3599
Jamapa	0.249	0.2797	0.06965	0.20661	1.0019	0.207003	0.3365
Protop-P1	0.282	0.2781	0.07842	0.25969	1.0003	0.259768	0.3019
FF4-13-MMMM	0.171	0.2688	0.04596	0.21153	1.0010	0.211742	0.2171
C-20	0.185	0.2225	0.04116	0.21750	1.0013	0.217783	0.189
Fleetwood	0.110	0.2735	0.03009	0.20859	1.0008	0.208757	0.1441
Mexico 12-1	0.201	0.2349	0.04721	0.22015	1.0007	0.220304	0.2143
Carioca	0.172	0.2455	0.04223	0.21837	1.0015	0.218698	0.1931
Laker	0.142	0.2316	0.03289	0.22907	1.0009	0.229276	0.1434
Black Magic	0.177	0.2576	0.04560	0.21808	1.0016	0.218429	0.2087
Swan Valley	0.103	0.2233	0.02299	0.24027	1.0009	0.240486	0.0956
Midnight	0.297	0.2003	0.05949	0.21735	1.0007	0.217502	0.2735
BAT 41	0.213	0.2373	0.05054	0.24602	1.0017	0.246438	0.2051
15-R-148	0.207	0.2593	0.05368	0.28461	1.0013	0.284980	0.1884
BAT 1507	0.271	0.2511	0.06805	0.23377	1.0001	0.233793	0.2911
BAC 95	0.106	0.2196	0.02299	0.23032	1.0011	0.230573	0.0997
Harblack (O)	0.160	0.2379	0.03806	0.20361	1.0008	0.203773	0.1868
Harblack (Sh)	0.252	0.2230	0.05620	0.21118	1.0019	0.211581	0.2656
Cumulus	0.229	0.2039	0.04669	0.22077	1.0013	0.221057	0.2112
N84004	0.103	0.2111	0.02174	0.24489	1.0016	0.245282	0.0886
C-20 Mutant	0.245	0.2090	0.05121	0.20676	1.0013	0.207029	0.2473
Jacob's Cattle	0.120	0.2325	0.02790	0.21700	1.0013	0.217282	0.1284
Huron	0.107	0.2314	0.02776	0.24234	1.0004	0.242437	0.1021
Mayflower	0.258	0.2040	0.05263	0.23675	1.0005	0.236868	0.2222
Albion	0.159	0.2229	0.03544	0.19849	1.0008	0.198649	0.1784
N87602	0.235	0.2073	0.04872	0.17161	1.0005	0.171696	0.2837
N78042	0.169	0.1598	0.02701	0.21062	1.0015	0.210936	0.1280
Huetar	0.233	0.2104	0.04902	0.21711	1.0016	0.217457	0.2254

Table 5. Indigestible protein in cooked beans in duplicate 'B'.

Identification	Protein in residue (g)	Residue from TDF (g)	Indigestible protein in residue (g)	Total protein flour (g)	Initial wt sample for TDF (g)	Total protein sample (g)	Indigestible protein (g)
N80242	0.147	0.1674	0.02461	0.20148	1.0011	0.201702	0.1220
Black Turtle Soup	0.184	0.2039	0.03752	0.20758	1.0013	0.207850	0.1805
Seafarer	0.264	0.2145	0.05663	0.20803	1.0003	0.208300	0.2719
Domino	0.267	0.2170	0.05794	0.24164	1.0017	0.242075	0.2393
Sanilac	0.089	0.2080	0.01851	0.21039	1.0013	0.210664	0.0879
Tuscola	0.143	0.1911	0.02733	0.24442	1.0007	0.244836	0.1116
8217-III-24	0.189	0.2172	0.04105	0.22445	1.0001	0.224472	0.1829
Jalpataqua	0.244	0.2172	0.05299	0.22891	1.0011	0.229162	0.2312
Nep-2	0.234	0.1517	0.03550	0.23408	1.0005	0.234197	0.1516
San Fernando	0.218	0.2091	0.04558	0.23699	1.0012	0.237274	0.1921
Bunsi	0.167	0.2155	0.03599	0.23019	1.0010	0.230420	0.1562
ICA Pijao	0.237	0.2296	0.05442	0.22689	1.0011	0.227140	0.2396
Aurora	0.191	0.2236	0.04271	0.23961	1.0019	0.240065	0.1779
P766	0.161	0.2126	0.03423	0.22626	1.0016	0.226622	0.1510
Jamapa	0.164	0.2473	0.04056	0.20661	1.0016	0.206941	0.1960
Protop-P1	0.160	0.2442	0.03907	0.25969	1.0013	0.260028	0.1503
FF4-13-MMMM	0.178	0.2350	0.04183	0.21153	1.0010	0.211742	0.1976
C-20	0.141	0.1955	0.02757	0.21750	1.0006	0.217631	0.1267
Fleetwood	0.093	0.2440	0.02269	0.20859	1.0015	0.208903	0.1086
Mexico 12-1	0.165	0.2140	0.03531	0.22015	1.0013	0.220436	0.1602
Carioca	0.131	0.2219	0.02907	0.21837	1.0007	0.218523	0.1330
Laker	0.104	0.2147	0.02233	0.22907	1.0006	0.229207	0.0974
Black Magic	0.171	0.2402	0.04107	0.21808	1.0019	0.218494	0.1880
Swan Valley	0.107	0.2441	0.02612	0.24027	1.0014	0.240606	0.1086
Midnight	0.158	0.1702	0.02689	0.21735	1.0007	0.217502	0.1236
BAT 41	0.177	0.2269	0.04016	0.24602	1.0004	0.246118	0.1632
15-R-148	0.188	0.2379	0.04473	0.27813	1.0008	0.278353	0.1607
BAT 1507	0.205	0.1997	0.04094	0.23377	1.0009	0.233980	0.1750
BAC 95	0.202	0.2149	0.04341	0.23032	1.0013	0.230619	0.1882
Harblack (O)	0.212	0.2243	0.04755	0.20361	1.0017	0.203956	0.2331
Harblack (Sh)	0.192	0.2213	0.04249	0.21118	1.0006	0.211307	0.2011
Cumulus	0.188	0.2378	0.04471	0.22077	1.0007	0.220925	0.2024
N84004	0.204	0.2291	0.04674	0.24489	1.0005	0.245012	0.1908
C-20 Mutant	0.185	0.2110	0.03904	0.20676	1.0008	0.206925	0.1887
Jacob's Cattle	0.148	0.2256	0.03339	0.21700	1.0018	0.217391	0.1536
Huron	0.171	0.2211	0.03781	0.24234	1.0014	0.242679	0.1558
Mayflower	0.164	0.1981	0.03249	0.23675	1.0015	0.237105	0.1370
Albion	0.159	0.2179	0.03465	0.19849	1.0008	0.198649	0.1744
N87602	0.131	0.1994	0.02612	0.17161	1.0015	0.171867	0.1520
N78042	0.154	0.1578	0.02430	0.21062	1.0014	0.210915	0.1152
Huetar	0.193	0.2559	0.04939	0.21711	1.0007	0.217262	0.2273

Table 6. Mean squares for the variable indigestible protein (g) for 41 dry bean genotypes prepared by two methods, raw and cooked.

Source of Variation	df	Indigestible Protein (g)	Pr>F
Duplication (D)	1	0.0177	0.0044**
Genotype (G)	40	0.0033	0.0394*
Duplication x Genotype	40	0.0739	0.6483 NS
Error	82		
Corrected Total	163		
CV (%)	28.9		

D = replications; G = genotypes

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively; NS = Non-significant

Table 7. Ranking of each raw bean genotype for indigestible starch on a per flour and a per starch basis.

Indigestible starch on a per flour basis			Indigestible starch on a per starch basis				
Genotype	Mean (mg/g flour)	Rank	Genotype	Mean (mg/100 mg starch)	Rank	Difference (di)	$(di)^2$
8217-III-24	21.5	1	8217-III-24	5.6	1	0	0
BAT 41	20.9	2	BAT 41	5.4	2	0	0
Huron	19.8	3	BAT 1507	5.2	4	-1	1
BAT 1507	16.4	4	Huron	4.4	3	1	1
Mexico 12-1	16.0	5	Mexico 12-1	3.9	5	0	0
Bunsi	15.7	6	Bunsi	3.7	6	0	0
BAC 95	14.3	7	Laker	3.7	8	-1	1
Laker	14.2	8	Black Magic	3.5	21	-13	169
Sanilac	14.0	9	15-R-148	3.5	11	-2	4
Albion	13.9	10	Mayflower	3.5	14	-4	16
15-R-148	13.7	11	Sanilac	3.5	9	2	4
Domino	13.6	12	Domino	3.4	12	0	0
Tuscola	13.3	13	Aurora	3.4	18	-5	25
Mayflower	13.3	14	BAC 95	3.4	7	7	49
Huetar	13.1	15	Huetar	3.4	15	0	0
ICA Pijao	13.1	16	Harblack (O)	3.3	26	-10	100
Black Turtle Soup	13.1	17	Jalpataqua	3.3	28	-11	121
Aurora	13.1	18	ICA Pijao	3.3	16	2	4
N84004	12.9	19	Black Turtle Soup	3.2	17	2	4
San Fernando	12.8	20	C-20	3.1	22	-2	4
Black Magic	12.4	21	N84004	3.1	19	2	4
C-20	12.4	22	Albion	3.1	10	10	144
Jamapa	12.2	23	Tuscola	3.1	13	10	100
Nep-2	11.3	24	San Fernando	3.0	20	4	16
Carioca	11.2	25	Jamapa	3.0	23	2	4
Harblack (O)	11.2	26	Carioca	2.9	25	1	1
FF4-13-MMMM	10.5	27	Fleetwood	2.6	29	-2	4
Jalpataqua	10.2	28	Nep-2	2.6	24	4	16
Fleetwood	10.2	29	N80242	2.6	30	-1	1
N80242	10.0	30	FF4-13-MMMM	2.6	27	3	9
Seafarer	10.0	31	Seafarer	2.5	31	0	0
Swan Valley	9.0	32	P766	2.5	33	-6	1
P766	9.7	33	C-20 Mutant	2.3	38	-5	25
N78042	9.5	34	N78042	2.3	34	0	0
Jacob's Cattle	8.8	35	Cumulus	2.2	36	-1	1
Cumulus	8.5	36	Swan Valley	2.2	32	4	16
N87602	8.5	37	Jacob's Cattle	1.9	35	2	4
C-20 Mutant	8.1	38	Protop-P1	1.9	41	-3	9
Midnight	8.1	39	N87602	1.9	37	2	4
Harblack (Sh)	8.0	40	Harblack (Sh)	1.8	40	0	0
Protop-P1	7.1	41	Midnight	1.8	39	2	4

Table 8. Ranking of each cooked bean genotype for indigestible starch on a per flour and a per starch basis.

Indigestible starch on a per flour basis			Indigestible starch on a per starch basis				
Genotype	Mean (mg/g flour)	Rank	Genotype	Mean (mg/100 mg starch)	Rank	Difference (di)	$(di)^2$
Cumulus	48.7		1 Cumulus		11.9		1
0	0						
N80242	44.9	2	N84004	10.3	5	3	9
Sanilac	41.9	3	BAT 41	10.1	4	-1	1
BAT 41	41.1	4	P766	9.8	24	-20	400
N84004	39.5	5	Black Turtle Soup	9.4	10	-5	25
Albion	37.1	6	N80242	9.0	2	4	16
C-20 Mutant	37.1	7	Huron	8.9	12	-5	25
Carioca	36.6	8	15-R-148	8.9	40	-32	1024
Laker	36.4	9	C-20 Mutant	8.7	7	2	4
Black Turtle Soup	35.3	10	8217-III-24	8.7	22	-12	144
Bunsi	34.9	11	Sanilac	8.5	3	8	64
Huron	34.6	12	BAC 95	8.5	14	-2	4
Seafarer	34.5	13	Domino	8.4	18	-5	25
BAC 95	34.3	14	Bunsi	8.4	11	3	9
Midnight	34.2	15	Aurora	8.4	31	-16	256
Jacob's Cattle	34.1	16	Seafarer	8.4	13	3	9
ICA Pijao	33.9	17	Huetar	8.3	19	-2	4
Domino	33.9	18	BAT 1507	8.3	21	-3	9
Huetar	33.6	19	Laker	8.3	9	10	100
C-20	33.4	20	Albion	8.3	6	14	196
BAT 1507	33.3	21	Protop-P1	8.2	25	-4	16
8217-III-24	33.1	22	Carioca	8.1	8	14	196
Nep-2	33.0	23	Midnight	8.1	15	8	64
P766	32.8	24	ICA Pijao	7.9	17	7	49
Protop-P1	32.4	25	Harblack (O)	7.9	29	-4	16
Mexico 12-1	32.4	26	C-20	7.9	20	-6	36
Fleetwood	31.7	27	Nep-2	7.9	23	4	16
N78042	31.7	28	Fleetwood	7.9	27	1	1
Harblack (O)	31.6	29	Jacob's Cattle	7.9	16	13	169
Tuscola	31.4	30	Harblack (Sh)	7.5	35	-5	25
Aurora	31.3	31	Jalpataqua	7.4	36	-5	25
Black Magic	31.1	32	N87602	7.3	38	-6	36
San Fernando	30.8	33	San Fernando	7.3	33	0	0
Swan Valley	30.8	34	Black Magic	7.3	32	2	4
Harblack (Sh)	30.3	35	Jamapa	7.2	37	-2	4
Jalpataqua	30.2	36	N78042	7.2	28	-8	64
Jamapa	29.6	37	Swan Valley	7.2	34	3	9
N87602	29.4	38	Mexico 12-1	7.0	26	12	144
FF4-13-MMMM	28.4	39	FF4-13-MMMM	6.7	39	0	0
15-R-148	28.0	40	Tuscola	6.7	30	10	100
Mayflower	27.0	41	Mayflower	6.3	41	0	0

Table 9. Spearman's rank correlation coefficient (r_s statistic) between the ranks of raw and cooked beans of 41 dry bean genotypes for indigestible starch on a per g of flour basis and on a per 100 mg starch basis.

	Spearman's rank correlation coefficient (r_s)	
	Raw	Cooked
Indigestible starch on a per g of flour basis vs. Indigestible starch on a per 100 mg of starch basis	0.9	0.7

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