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STUDIES ON THE INDIGESTIBLE AND FERMENTABLE COMPONENTS OF DRY BEAN AND CEREAL GRAINS

By

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ABSTRACT

STUDIES OF THE INDIGESTIBILITY AND FERMENTABILITY
OF DRY BEANS AND CEREALS

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Parents and progeny of an 8 X 8 diallel cross were evaluated to ascertain if genetic variability for low molecular weight sugars in beans, namely sucrose, raffinose and stachyose existed. The various sugars were extracted using 80% ethanol 20% water and analyzed on an Alltech 600 CHC carbohydrate column using an elution solvent of acetonitrile:water 70:30 (v/v). Data was subjected to a randomized complete block design using a 2 way-analysis of variance with sampling and determinations. Results showed significant and highly significant differences among the parents for raffinose and stachyose respectively. Sucrose was nonsignificant as were the sampling and determination mean squares. In the 56 crosses sucrose, raffinose and stachyose were significant at the 1% level. The sampling and determination mean squares were nonsignificant.

Samples of dry beans (Phaseolus vulgaris) and controls of cereals were digested in-vitro for the removal of digestible protein and starch. The remaining residue (indigestible residue) was analyzed for indigestible protein, starch, soluble sugars and the various fiber components. Protein residue remaining after digestion for the samples varied from 16.1% for 'Seafarer' to 29.5% for 'San Fernando'. After digestion protein found in the cereal samples varied from 5.8% for pearled barley to 14.5% for corn. Indigestible starch content of the

dry bean samples were from 13.7% for 'Seafarer' to 21.4% for 'Fleetwood'; while in the cereals, values varied from 10.8% for corn to 19.0% for whole wheat. The dry bean samples contained between 2.18 to 2.94% stachyose and 0.34 to 0.53% raffinose. Raffinose and stachyose were not observed in the cereals. Dietary fiber content for the dry bean samples varied from 17.8% for 'UI-114' to 19.9% for 'Sanilac' and 'Proseed'. In the cereal samples, pearled barley had the highest dietary fiber at 14.8% and corn the lowest at 8.6%.

Each component and the total of the components found in the indigestible residue were estimated for the amount that would be theoretically fermented in the colon and the volume of gas that would be produced upon fermentation. The percent of the sample fermentable based on the original sample weight for dry beans ranged from 24.7 mg/100 mg for 'Nep-2' which could produce 5.3 ml of gas to 27.3 mg/100 mg for 'Proseed' which could produce 6.0 ml of gas. The cereals ranged from 9.9 mg/100 mg for oats which could produce 2.4 ml of gas to 6.1 mg/100 mg for pearled barley which could produce 4.0 ml of gas.

When 100 mg of the indigestible residues were actually fermented the total volumes of gas produced from the dry bean samples ranged from 33.0 ml for 'Nep-2' to 24.0 ml for 'Seafarer'. The rates of fermentation ranged from 0.74 ml per hour for 'Proseed' to 0.41 ml per hour for 'Seafarer'. In the cereals wheat and barley produced 27.0 ml while oats produced 26.2 ml. The rates of fermentation ranged from 1.56 per hour for oats to 1.37 per hour for wheat and pearled barley.

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INTRODUCTION

Legumes are an important human food on a world basis. Although dry beans are a good source of protein of reasonable quality, their annual per capita consumption in countries such as United States, Sweden and Argentina is only 1.1 to 5.4 Kg per year. This is in sharp contrast to countries such as India, Brazil, Mexico the United Kingdom and Japan where annual per capita consumption is 18 to 36.5 Kg (USDA, 1979). However the world wide per capita consumption of legumes is steadily decreasing.

One of the major reasons for the avoidance of legumes is its potential to create abdominal discomfort, dyspepsia, diarrhea, nausea and the production of gas which can give a bloated feeling and a social embarrasssment due to the elimination of intestinal gas (flatulence). Although many foods produce significant amounts of intestinal gas, the largest flatus volumes have been attributed to seeds of edible grain legume (Hickey et al., 1972).

In the developed countries the social embarrassment of the egestion of noxious gasses hinder many from using legumes in their diets. The relatively low status of eating food legumes in the United States is in contrast to the broad U.S. nutritional guidelines of decreasing total fat and cholesterol consumption and increasing complex carbohydrate consumption. These guidelines could be accomplished by decreasing the consumption of protein derived from animal sources and increasing the consumption of legumes. Also, it would be financially advantageous for elderly individuals on fixed

incomes to choose the lower priced protein sources such as legumes rather than the higher priced protein sources such as meat and diary.

But in lesser developed countries, the maintainence of a balanced nutritional diet by the mixture of protein from different food sources, such as legumes, is essential in providing good health. This is especially true for infants and children which generally receive inadequate protein nutrition. However, the current trend is for mothers in developing countries to discourage the inclusion of legumes in diets of infants and children rather than to increase the use of legumes in their diets.

It is widely recognized that legume consumption would increase if digestibility of the grains could be increased and the associated flatulence difficulties could be overcome. However, there is no methodology available to bean breeders for identifying and selecting strains with good digestibility and low flatulence potential. Consequently, this thesis research was to elcidate factors resposible for gas production and assess the feasibility of characterizing the constituents responsible.

There is a need to characterize the indigestible residue from beans to provide a clearer picture on the gas forming components. In addition there has been relatively little research concerning ways to remove the soluble sugars (implicated in causing intestinal gas) by technological and/or genetic intervention. In view of the need for further research in the area of indigestibility of seed components and resultant gas production from eating beans this thesis study was initiated. The specific objectives were to: evaluate parents and progeny of an 8 X 8 diallel cross to ascertain the magnitude of

genetic variability for low molecular weight sugars namely sucrose, raffinose and stachyose in beans 2) Characterize indigestible residue in bean and cereal grains, and compare the theoretical and actual amounts of gas produced to elucidate the causal components of flatulence, and 3) develop a method useful to breeders for screeing low flatulence producing beans.

REVIEW OF LITERATURE

An understanding of the causes of flatulence is related to the fate of carbohydrates, proteins, and lipids in the digestive tract.

Digestion

Overview of the Gastrointestinal tract and its function. The gastrointestinal (GI) tract consists of the mouth, pharynx, esophagus, stomach, small intestine and colon. Absorption of simple molecules occurs at the microvilli surface of the duodenum, jejunum and ileum of the small intestine. A high concentration of hydrolytic enzymes in the microvilli of the small intestine aid in the absorption process.

Chemical constituents that are not absorbed in the small intestine enter the colon where they transit slowly and are eliminated. Movement in the colon is dependent on the volume of nonabsorbable (undigested) material (Spiller and Amen, 1976). The movement from stomach to the end of the ileum may require 30 to 90 minutes; while passage through the colon may require 1 to 7 days. Bacteria are abundant (often in excess of 10^8 bacteria per ml of intestinal content) in the colon. These bacteria metabolize foods not broken down by the human digestive tract. The break down of simple and complex polysaccharides by colonic bacteria leads to the production of hydrogen, carbon dioxide, and short chain fatty acids. Nondigestible protein residues are altered by bacteria to odoriferous molecules, namely skatole from tryptophan and mercaptans from sulfur-containing amino acids. The undigested fatty acids are partially metabolized causing a stimulation of colon secretion (Bright-Asare

and Binder, 1973).

Carbohydrate digestion. The digestion of dietary carbohydrates can be broken into subgroups that are digested in slightly different ways. These subgroups are starches, oligo- and disaccharides, and monosaccharides. Since only small molecules can be absorbed by the small intestine, certain enzymes are present in the GI tract to hydrolyze complex molecules into smaller components.

Starch is a linear chain of glucose molecules in α 1-4, or α 1-6, linkages. Starch is primarily digested at the duodenum by a-amylase secreted by the pancreas. This enzyme cleaves to the interior links of the polysaccharide. The end products of starch digestion are maltose, maltotriose and a limit dextrins. These products move to the intestinal surface where surface enzymes hydrolyze them further into monosaccharides which can be transported across the microvilli membranes.

The oligosaccharides and disaccharides must also undergo hydrolysis at the outer surface of the brush border membranes before absorption can occur. Monsaccharides do not require digestion prio to absorption. Carbohydrates that cannot be hydrolyzed because of a lack of specific enzymes for their breakdown are called dietary fiber. This fiber passes through the small intestine to the colon. Dietary fiber has been associated with material from plant origin and consists of lignin, cellulose, hemicellulose and pectic substances. Dietary fiber should also include indigestible oligosaccharides such as raffinose, stachyose and verbacose.

Protein digestion. The digestion of protein begins in the stomach and is brought about by the secretion of pepsin and gastric juices. Once the protein leaves the stomach, it is further hydrolyzed by

proteolytic proenzymes secreted by the pancreas and proteolytic enzymes embedded in the microvilli membrane and within absorptive cells of the mucosa of the small intestine. The secretion of the pancreatic enzymes apparently is regulated by the presence of dietary protein in the small intestine (Ochoa-Solano and Gilter, 1968). The end-products of protein digestion (amino acids, di- and tripeptides) are absorbed into the mucosal cells of the small intestine.

Lipid digestion. Most dietary fats are in the form of triglycerides (glycerol esters of fatty acids). The glycerol components are constant in structure while the fatty acids may consist of a long or short chain, and be saturated (presence of hydrogen) or unsaturated (absence of hydrogen). The fatty acids or their fatty acid derivatives will be utilized as a source of energy.

The fatty acids are highly water insoluble molecules making it difficult to move across the unstirred layer of fluid that is entrapped by the microvilli of the gut lining. To overcome this problem the fat emulsion, which is free from protein by stomach proteolytic enzymes and starch by salivary amylases, enters the duodenum where it is mixed with bile and pancreatic lipase. The bile salts are highly polar and have water soluble groups attached to the outside of the molecule. The bile salts will aggregate in water solution forming micelles. Due to this property the micelles trap large concentrations of lipid soluble materials internally and can diffuse more rapidly now through the unstirred water layer because of the water soluble groups of the bile salts. This transport mechcanism increases the concentration of lipid to the surface of the mucosa cells which would otherwise be a slow diffusion process.

The fatty acids and monoglycerides are then absorbed through the mucosa and the bile salts reenter the lumen to be recirculated through the liver for reuse in the intestine.

Composition of Legume Seeds

Digestible carbohydrates. The carbohydrate content of dry legume seeds can range from 24.0% in winged beans to 68.0% in cowpeas (Reddy et al., 1984). Table 1 shows the quantitative composition of various legumes with regard to their carbohydrate fraction.

The starch components amylose and amylopectin are a linked glucose units which cause formation of helix structures, and are of a water soluble nature. They are able to be hydrolyzed by mammalian enzymes. Starch is the most abundant legume carbohydrate and can vary from 56% in dry beans to 0.2 to 3% for soybeans; variations are due to both cultivars and the analytical procedures used (Pritchard et al., 1973; Cerning et al., 1975). The disaccharide sucrose is also found in legumes ranging from 2 to 3%.

Indigestible carbohydrates. The oligosaccharides represent only a small amount of the total carbohydrate content of dry legume seeds (Table 1). Raffinose, stachyose, and verbascose account for most of the sugars. Verbascose is the major oligosaccharide in black gram, Bengal gram, red gram, mung bean and faba beans. Stachyose is the major oligosaccharide in smooth and wrinkled peas, soybeans, and most commercial classes of dry beans (Nene et al., 1975; Hymowitz et al., 1972; Cerning-Beroard and Filiatre, 1976; Naivikul and D, Appolonia, 1978; Reddy and Salunkhe, 1980; Fleming, 1981; Sathe and Salunkhe,

Table 1. Proximate composition of dry bean carbohydrates.

Composition	Red kidney beans	Navy beans	Pinto beans	Pink beans
Total carbohydrate	56-60	58	54-63	--
Starch (%)	31-47	27-53	51-56	42
Amylose (%)	18-37	22-36	25	15-35
Soluble sugars				
Sucrose (%)	2	3	3	1.4
Raffinose (%)	0.3-0.9	0.4-0.7	0.4-0.6	0.2-0.4
Stachyose (%)	2.4-4.0	2.6-3.5	2.9-3.0	0.2-0.4

Source: Reddy, N.R. et al. 1984. Chemical, nutritional and physiological aspects of dry bean carbohydrate-A review.

1981).

In the literature the fiber portion of the legumes has a complicated and inconsistent nomenclature and is referred to as dietary fiber or unavailable carbohydrate, indigestible residue (IR) or crude fiber (CF). Table 2 shows a comparison of the nomenclature and the chemical or enzymatic methods for analyses. The differences between crude fiber and indigestible fiber are that a high percentage of hemicelluloses and all of the pectins and gums are not included when measuring crude fiber. These components are easily destroyed by strong oxidative treatment or they are soluble in hot liquids and lost during the fiber extraction process.

Trowell (1974) postulated a physiological concept of fiber which is called dietary fiber and is defined as the remnant of plant cell walls resistant to hydrolysis by the enzymes of alimentary tract of man. This definition was later expanded to include undigested storage polysaccharides present within the contents of the cell (Trowell et al., 1976). These definitions caused problems because they excluded the following compounds: 1) structural protein, extensin (hydroxyproline-hyp), bound in the cell walls by specific polysaccharides (Preston, 1974), 2) indigestible animal connective tissues and 3) endogenous acidic mucopolysaccharides (mucus) from the alimentary tract which have been shown to have a fiber-like action (Godding, 1976).

Williams and Olmsted (1935) and Hellendoorn (1975) disfavored the term dietary fiber since it was closely associated with the botanical concept of polysaccharides which are undigestible by man. These authors favored the term "indigestible residue" which reflected the

Table 2. Nomenclatures of Fiber.

Unavailable carbohydrates (and lignin)	By "difference", subtraction from 100 the percent water, starch, soluble sugars, protein, fat and ash (McCane and Widdowson, 1960).
	By chemical analysis of the components (Southgate, 1969; Van Soest and McQueen, 1973).
Crude fiber	By Chemical Hydrolysis a) alternating treatment with alkali and acid. (Weende Method, Horwitz, 1970).
	b) oxidative treatment (Scharrer-Kurschner-Van de Kamer and Van Kenkel, 1952).
Dietary fiber or Indigestible food Residue	By treatment with alimentary or bacterial digestive enzymes (Willims and Olmsted, 1935; Hellendoorn, 1975; Asp, 1983).

Source: Hellendoorn, E. 1978. Fermentation as the principal cause of the physiological activity of indigestible food residue. In: Topics in dietary fiber research. p 127. Plenum Press, New York.

process taking place in the small intestine and is nonspecific to a class of materials.

Of the polysaccharides comprising dietary fiber (Table 3), cellulose is the main structural polysaccharide in plants. It is a linear polymer of linked glucose with a high degree of polymerization (D.P.) of up to 10,000 (Theander 1976). The molecules have a strong intermolecular and intramolecular hydrogen bonding causing the formation of microfibrils, which give it high mechanical strength and resistance to hydrolysis by chemicals.

The hemicelluloses consist of xylans and glucomannans and have a close chemical structure to cellulose. These classes of polysaccharides are based on their β-1-4-linked sugar chains. Here the pentose D-xylose differs from D-glucose by the absence of the C-6 CH_2OH unit and the hexose D-mannose differs only in the configuration of C-2. The xylans have the basic β-linked D-xylose units with singly attached units of l-arabinose and/or D-glucuronic acid or its 4-O-methyl esther while the glucomannans will have mannose units predominating throughout the chain (Theander 1976).

Pectic substances or pectins are amorphous polysaccharides found universally in the primary cell walls and intercellular layers in land plants. One group, galacturonans, consists largely of unbranched chains of β-1,4 linked units of D-galacturonic acid units which may contain L-rhamnose, D-galactose and L-arabinose units (Theander, 1976).

Lignin is a very complex, branched polymer that originates from phenylpropanoid units, interconnected in varying proportions and random sequences in various ways. It is believed that lignin is

Table 3. Dietary Fiber.

Type	Chemical Characteristics	Plant Cell Function
Cellulose	Unbranched 1-4 β-D glucose	Cell wall
Hemicellulose	Pentose or hexose polymers often branced; soluble in cold alkli	Wall stability
Pectins	β-1, 4-D-galatcuronic acid polymers usually associated with other polysaccharides	Wall stability
Lignin	Noncarbohydrate substituted phenylpropane	Cell wall strength

Source: Gray, G. and Fogel M. 1980. In: Modern Nutrition in Health and Disease. p. 99. Lea & Febiger, Philadelphia Penn.

partly linked to polysaccharide cell-wall components (Adler 1961).

Table 4 shows that legumes contain appreciable amounts of fiber (1.2 to 13.5%) with large variations observed. The hemicelluloses are the major fiber component in legumes but vary widely within the legume family. Hemicellulose of horse beans contain essentially xylose and small amounts of arabinose. Sagjan and Wankhede (1981) found that the hemicellulose fraction of winged beans consisted of glucose, xylose and arabinose of 15.5:9:1 in one fraction and glucose and xylose in the proportion 15:1 in the other fraction. Little work has been done in the isolation of hemicellulose components in P. vulgaris.

Protein. Data on nutrient composition of several species of dry beans shows little variation in protein content of beans belonging to the same species. Table 5 compares protein (and other components) of dry P. vulgaris with the components of cereals. Legumes overall have a high protein content averaging between 20% to 25% on a dry weight basis. The average protein content of legumes is twice the amount found in cereal crops on a per serving basis (Bressani, 1975).

Proteins present in seeds of legumes are of two types: 1) metabolic proteins (enzymatic and structural) which are responsible for normal cellular activities including the synthesis of structural proteins, and 2) storage proteins. These storage proteins are synthesized during seed development and occur within the cell in discrete protein bodies.

The predominant class of storage protein present in the Phaseolus seed is the salt soluble globulin 1 fraction which accounts for 40% of the total cotyledon protein. The alkali-soluble fraction which accounts for 25% of the cotyledon protein, contains glutelin and

Table 4. Fiber content of dry beans.

Legume	Crude Fiber %	Lignin %	Cellulose %	Hemicellulose %
Navy	3.4-6.6	0.1	3.2	0.5-4.9
Red kidney	3.7	2.7-3.1	2.5-5.9	0.3
Pinto	4.3-7.2	1.8-3.0	9.0	4.0
Black eye	--	0.1	4.9	--
Smooth pea	4.6-7.0	0.5-0.9	0.0-4.9	1.0-5.1
Wrinkled pea	7.6	0.3-1.0	1.2-4.2	0.9-6.6

Source: Reddy, N.R. et al. 1984. Chemical, nutritional and physiological aspects of dry bean carbohydrate-A review.

Table 5. Composition of dry beans and cereals - Major components.

			% dry basis			
				-carbohydrate-		
Legume	Moisture	Protein	Lipid	Total	Fiber	Ash
Navy	10.9	25.0	1.7	69.0	4.8	4.4
Pinto	8.3	25.0	1.3	69.0	4.8	4.2
Black eye	11.2	25.0	1.3	69.0	5.0	4.3
Wheat	14.0	12.0	2.3	83.8	2.7	2.0
Barley	11.1	9.2	1.1	89.0	0.6	1.0
Oats	8.3	15.5	8.1	74.0	1.3	2.1
Corn	13.8	10.3	4.5	84.0	2.3	1.4

Source: USDA Handbook (1977)

albumin, free amino acids, globulin-2, prolamine, and residue fractions (Ma and Bliss, 1978; Bressani, 1975; Kay, 1979).

Nutritional Digestibility of Legume Carbohydrate and Protein

Carbohydrate digestibility in-vitro. Most of the in-vitro studies of carbohydrate digestibility are based on the amount (mg) of maltose released per 100 mg of flour after amylolysis for specified periods of time. The enzyme -amylase, isolated from porcine pancreas, bacteria, and malt, is used in most in-vitro studies (Greenwood and Thomson, 1962; Elbert and Witt, 1968; Kakade and Evans, 1966; Borchers, 1962; Rao, 1969). Starch isolated from legumes differs in its in-vitro digestibility when comparing hog pancreatic α-amylase or bacterial α-amylase (Borchers, 1962; Rao, 1969, 1976).

Mature bean seeds are less digestible than immature seeds. This could result from changes in the composition of starch and non-starchy components during maturation (Greenwood and Thomson, 1962; Elbert and Witt, 1968). A significant increase in te in-vitro carbohydrate digestibility was observed following the boiling of whole legume seeds. Enhanced digestibility of cooked legume starches by α-amylase could be attributed to 1) the swelling and rupturing of starch granules, 2) the disintegration of various bean components during cooking, and 3) inactivation of a amylase inhibitors.

In-vitro studies have shown that nongerminated legumes are less digestible than germinated (bean sprouts) or processed ones. This increase in digestibility of the germinated and processed beans was thought to be due to the leaching of protease inhibitors during soaking and rinsing preparation (Kakade and Evans, 1966).

Geervani and Theophilus (1981) measured the digestibility of

legume carbohydrates (red and Bengal gram (Cajanus) and black gram and green gram (Phaseolus)), by in-vitro and in-vivo techniques. By these methods Phaseolus was found to be superior to the Cajanus genus. With all samples, boiling and pressure cooking improved digestibility compared to roasting. The digestibility of fermented and germinated legumes was not much higher than legumes processed by other methods. It was noted that there was no correlation between in-vitro and in-vivo carbohydrate digestibility. The reasons for the lack of correlation were due to product inhibition during the in-vitro studies, i.e. accumulation of maltose, isomaltose and glucose slows down the rate of hydrolysis by α-amylase, glucoamylase and maltase in the in-vitro studies.

Carbohydrate digestibility in-vivo. Rao (1969) studied in-vivo digestibility of carbohydrates in 3 and 4 year old children by measuring blood glucose content. Peak blood glucose levels were reached with green gram at the end of 30 minutes. Blood glucose levels peaked in 60 minutes for Bengal gram. These findings support the in-vitro observations that carbohydrates in green gram are more rapidly digested and more easily available than those in Bengal gram.

Fleming & Vose (1979) and Fleming (1982_a) investigated the in-vivo digestibility of starches in legumes by measuring the starch content in the rat cecum. They compared legume starch digestibility with that of wheat which was 100% digestible. Starches from all legumes, excluding the high amylose wrinked pea, were nearly 100% digestible, but the legume starches reduced the digestibility of casein protein by 3 to 4%.

Wong et al. (1985) investigating the extremely slow rate of

digestion and absorption of starch from legumes on a in-vitro level found that the rate of starch hydrolysis was not affected by the presence of fat (as either butter or an emulsion) or the viscosity of the slurry. Significant increases in the rate of starch hydrolysis was observed in commerically available canned bean preparations (suggesting that the high temperatures used in canning may alter the availability of the starch) and by grinding the legumes finely prior to cooking.

Karimzadegan et al. (1979), using slope-ratio analysis of weight gain and plasma ketones of rats, found that the apparent availabilities of the carbohydrates for digestion in soybean meal, lima bean, and Bengal gram, respectively are 35, 70 and 80%.

The differences in digestibility of various starches have been attributed to many factors. Higher amylose content starches have been shown to be lower in digestibility. Other factors such as degree of polymerization, microheterogeneity or botanical sources of starch may affect digestibility. The presence of non-starchy components, such as protein, lipids, cellulose, hemicellulose and galactose-containing oligosaccharides may hinder hydrolysis or block absorption of the hydrolyzed starch. The nature of the digestive enzyme in-vitro or inhibition of the enzyme by a amylase inhibitors will affect the degree of digestiblity of starch (Hellendoorn 1972, 1978; Biliaderis et al., 1981).

Protein digestibility. In-vivo digestibility of common bean proteins has been reported to range from 65 to 88%. Vaintraaub et al. (1975) denatured and digested the P. vulgaris protein with both pepsin and trypsin and found that about 5.6% of the protein remained un-hydrolyzable. Rockland (1978), using in-vitro techniques, found a

median of 77% with four cooked commerical types of dry legumes, compared to 93% for a casein standard. Digestibilites of the legume were consistently 15-20% lower than that of casein.

Low digestibility of legume protein has been observed to vary not only among legume species but also among varieties of the same species (Bressani, 1973). Protein digestibilities varied from 44 to 84% for different varieties of P. vulgaris (Jaffe, 1973; Kakade, 1974; Pusztai et al., 1975).

Fractionation studies of kidney beans by Pusztai et al. (1975) showed that the globulin (37.3% of total nitrogen) and albumin (25.7% of total nitrogen) fractions had true digestibilities of 76 and 82%, respectively in a rat bioassay. In in-vitro studies the globulin fractions from P. vulgaris (which comprises a large part of the bean seed protein) was associated with low digestibility when a number of proteolytic enzymes were used even after thermal and urea treatments (Jaffe and VegaLette, 1968; Seidel et al., 1969). Pusztai et al. (1975), using P. vulgaris also found that about 30% of the albumin fraction and 10% of the globulin fraction only showed a 10% trypsin breakdown after 24 hours compared to casein which was completely digested in one hour. Lectins from P. vulgaris also have proved to be resistant to digestion by pepsin and proteolytic bacteria (Jaffe and Hanning, 1965; Jayne-Williams and Burgess, 1974).

Low solubility and digestibility of legume proteins under physiological conditions may be due to highly organized, tightly folded, compact structures which prevent hydration of protein molecules and protect peptidase-susceptible bonds from enzyme attack. This protein conformation may give a measure of resistance to

proteases of the alimentary canal (De Muelenaere et al., 1961; Chen et al., 1962).

Tannins. Condensed polyphenols in beans are mainly tannins and are found mainly in the testa. Jaffe (1950) suggested there may be a relationship between protein digestibility and seed-coat color in P. vulgaris. This implicated the roles of tannins and other polyphenols. Colored seeded beans contain tannins while white seed coat beans are devoid of tannins (Ma & Bliss, 1978). Cooking has been shown to have little effect on destroying tannins. However, they are partially removed with the cooking broth. The effect of tannins on protein digestibility is possibly due to the formation of soluble and insoluble complexes which are poorly digestible and or unavialable (Van Buren and Robinson, 1969; Feeney, 1969). Furthermore, tannins have been shown to be thermo-stable, that is not inactivated by heat treatment. Tanins inactivate a-amylases and lipases (Tamir and Alumont, 1969; Milic et al., 1972). Griffiths and Mosley (1980) assayed the saline extract of intestinal contents of rats for trypsin, amylase and lipase. The activities of both trypsin and amylase were signifcantly reduced in rats consuming diets containing the testa from colored flowering field beans. On the other hand, rats fed testa from white flowering beans without tannins had comparable activities with those fed a control diet.

Phillips and Eyre (1981), using low and high polyphenol beans measured true digestibility and biological value of cooked meals of two high polyphenol cultivars and one low-polyphenol cultivar using nitrogen balance experiments with growing rats. Removal of the testa caused an increase in true digestibility of the high polyphenol beans (7.2 to 88.4%), but not of the low polyphenol beans. Testa removal

caused no change in biological value. Polyphenols probably decrease protein digestibility in humans by making proteins partially unavailable or by inhibiting digestive enzymes.

Flatulence

Suspected causes. Any material that is not digested and absorbed in the small intestine is available for microbial digestion in the colon with subsequent flatus production. Establishing a cause and effect relationship between dietary factors and flatulence has been long and arduous.

Alvarez (1942) suggested that swallowed air or the diffusion of gasses from the blood into the intestinal lumen were responsible for flatus. Based on the high carbon dioxide content of flatus, Danhof et al. (1963) proposed that secretions in the intestine (especially the pancreatic secretion) may be the causal factor of flatus. But as early as 1918, Kanto (1918) linked bean diets and gas production to bacterial fermentation within the lumen of the intestine.

The first suggestion that carbohydrates in dry beans may in part be responsible for flatus was made by Anderson (1924). He was able to demonstrate that the primary gasses evolved in anaerobic fermentation of various carbohydrate containing media were high percentages of carbon dioxide and hydrogen and low percentages of nitrogen and oxygen.

Olson et al. (1975) and Van Stratum & Rudrum (1979) reported that the protein rich fractions from California Small White beans (CSW) and soybeans did not significantly contribute to flatulence in rats and humans.

Raffinose and Stachyose. Steggerda et al. (1966) found that when human subjects consumed various fractions of soybean meal that the low molecular weight carbohydrate fractions were especially potent in gas production compared with fat, protein, and complex polysaccharide fractions. Steggerda and Dimmick (1966) showed that differences in human flatus production and gas composition occurred with different kinds and quantities of bean diets. They observed the average concentration of carbon dioxide in the collected flatus changed from 11% on a controlled non-gas producing diet to 51% when large quantities of pork and beans were consumed.

The oligosaccharides raffinose, stachyose and verbascose are found with dry legumes. Raffinose a trisaccharide, contains galactose, glucose and a fructose molecule with the galactose moiety having a 1-6 bond. Stachyose a tetrasaccharide, is made up of two adjacent galactose units, one glucose and one fructose with the similar 1-6 bond. Verbascose a pentasaccharide, is made up of three adjacent galactose units, one glucose and one frctose with the similar 1-6 bond. Calloway (1966), subjected stachyose to fermentation with colonic bacteria. She found that the bacteria had the capability to hydrolyze the 1-6 galactoside of stachyose. No 1-6 galactosidase has been found in mammals (Gitzelmann & Ajurricchio, 1965) and the raffinose family sugars are unable to pass through the intestinal wall (Cristofaro et al., 1973). This led to speculation that sugars of the raffinose family (raffinose, stachyose and verbascose) may cause much of the intolerance associated with beans. These sugars are not absorbed due to the presence of this 1-6 bond and their fate is left to the bacteria in the lower GI tract where they would be metabolized into 2 and 3 carbon fragments and large amounts of gas.

Cristofaro et al. (1973) reported that diets containing stachyose and verbacose exhibited the highest flatus activity. Rackis et al. (1970), using an in-vitro assay, showed that toasted, dehulled, and defatted soybean meal had gas producing factors of sucrose, raffinose and stachyose and gas-inhibiting factors of phenolic (syrigic and ferulic) acids. The lipids, proteins and water-insoluble polysaccharides of soybean meal had no gas activity.

Murphy et al. (1972) used human subjects and the California Small White bean. The bean was treated by extraction using 60% and 85% aqueous ethanol. Results showed that the flatulent activity was associated in the 60% and 85% aqueous extracted ethanol phase which contained raffinose and stachyose. But raffinose and stachyose fed alone at levels found in California Small White bean did not increase the carbon dioxide level of the flatus. The sugars made a major contribution to the hydrogen component of the breath and flatus during the period of high flatus volume; but the major factors responsible for the increase in carbon dioxide volume remained unidentified.

Fleming (1981), using rats, showed a significant positive correlation between hydrogen production and 1) stachyose, 2) raffinose plus stachyose, and 3) glucans and pentosans which were hydrolyzable in dilute acid.

Dietary fiber. Recent studies indicated that even after the removal of oligosaccharides, dry beans could still induce appreciable flatus (Olseo et al., 1975; Fleming, 1981, 1982_b; Hellendoorn, 1976, 1979; Kamat & Kutkarni, 1981). Wagner (1976) found that when men and rats were fed similar diets a positive correlation existed between breath hydrogen production and flatus gas volumes. Wagner (1977) then

compared a cooked CSW bean which contained 4% oligosaccharides with an oligosaccharide free CSW solids preparation (residue from hexane and 70% ethanol extraction of CSW). The oligosaccharides served as a source of hydrogen gas when ingested by rats in life support systems. Wagner showed that if the oligosaccharide contents were the only hydrogen source in CSW, it would have had to be 25 times as potent as CSW, but raffinose was only five times and stachyose seven times as potent as CSW. Thus CSW contained at least one 70% alcohol-insoluble substance which in addition to the oligosaccharides was essential to bring about the quantitative physiological response to whole beans observed in rats.

Dietary fiber, defined as indigestible dietary components, has been shown to be fermented by microorganisms in the colon. Analysis of feces for the prescence of fiber has shown the greater part of cellulose and lignin can be recovered in the stool (Southgate and Durnin, 1970; Williams and Olmstead, 1936a; Williams and Olmstead, 1936b). Holloway et al. (1980, 1983) measured the digestibility of pectin and hemicellulose in humans on an input/output basis. Of the subjects fed pectin diets, 15.3% of the pectin was digested by women ileostomy subjects and 46.5% was digested by men ileostomy subjects; while 96.5 and 95% of the pectin was digested by men and women subjects with intact ileums. When subjects were fed hemicellulose diets, 65% of the hemicelluloses were digested by women ileostomy subjects and 63% by male ileostomy subjects. On the other hand, 95 and 97% of the hemicelluloses were digested by men and women subjects with intact ileums. The possibility of bacterial growth within the ileostomy bags or altered bacterial flora in the small intestine were stated as reasons for hemicellulose and pectin loss in the ileostomy

subjects.

Prynne and Southgate (1979) found a large range for apparent digestibility of total fiber from 47 to 82% during the experimental period using human subjects fed a strictly controlled diet of a fiber supplement. During the control period values of 70 to 80% for the apparent digestibility of the fiber were observed.

Farrell et al. (1978) fed human subjects neutral detergent fiber and observed a complete disappearance of hemicellulose from diets with increased transit time, while 80% and 55% of the neutral detergent fiber in the diet disappeared from low and high fiber diets respectively.

Fermentation of fiber and passage to the large intestine were monitored by measuring hydrogen gas (H_2) concentration in expired breath (Hanson et al., 1985). Breath samples were obtained at 30 minute intervals for 9 hours after the test meals. The subjects were fed 40 gram meal samples. The number of hours from the meal to the highest H_2 peaks were 4.7 for wheat bran, 5.6 for corn bran, 6.2 for oat bran, 6.4 for citrus flour and 8.2 basal.

Fleming (1981) showed a significant positive correlation between hydrogen production and the dietary content of glucans and pentosans which are hydrolyzable in dilute acid. Significant negative correlations were found between hydrogen production and starch or lignin contents. Marthinsen and Fleming (1982) measured the breath and flatus gasses of humans consuming high-fiber diets and found xylan and pectin diets resulted in a production of high flatus volume. Cellulose and corn bran generally produced breath hydrogen and flatus gas excretion at levels equivalent to a fiber-free diet.

Hellendoorn (1978) found that while most of the hemicelluloses and soluble pectins were fermented in the large intestine, some could be recovered from the stool. Tadesse and Eastwood (1978) reported a hemicellulose preparation increased hydrogen production in man, but cellulose, lignin, and pectin did not. Fleming (1981), using the smooth seeded field pea, found hydrogen production in the rat to be closely associated with the quantity of oligosaccharides remaining in the seed meal. However, the flatulent acuivity of the whole seed appeared to be due in equal parts to the indigestible oligosaccharides and components of the cell-wall fiber.

Fleming (1983) compared the effects of selected purified fibers to those derived from cereals or legume seeds. Rats were fed diets containing 10% dietary fiber and 10% protein. Pectin reduced rat weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER) and apparent protein digestibility values compared to a fiber-free diet. Cellulose, xylan and raffinose had no influence on feed intake, weight gains or FER. Cellulose and xylan increased PER values and the rates of food passage through the gastrointestinal tract but decreased the apparent protein digestibility values. Finally, the cell-wall-fiber fraction of beans had little effect on feed consumption, growth, FER or PER. The cell-wall-fiber fraction of the beans reduced apparent protein digestibility and the hull fraction accelerated food passage relative to the fiber-free diet.

Starch. It is feasible that normally digestible carbohydrates (such as starch) may not be completely digested if the organism lacks sufficient digestive capacity relative to the amount or type of carbohydrate ingested. This may be due to consumption of carbohydrates which are less accessible or resistant to hydrolysis, a

deficiency in the hydrolyzing capacity of the individual (inherited or temporary), the result of insufficient reaction time to digest and absorb the carbohydrate properly, or the result of a too rapid food transit (Hellendoorn 1978).

It has been observed that starch in bread, upon becoming stale, is less easily digested in-vitro by salivary or pancreatic amylolytic enzymes (Jackel and Schraeder, 1953). Dry navy beans harvested the year prior to analysis and kept under unfavorable temperature and humidity conditions showed that 10 to 15% of the starch remained indigestible with in-vitro measurements.

Stephen (1983) using mixtures of starch from navy beans, rice and potatoes, directly measured the passage of carbohydrates through the small intestine by inserting an aspirator at the human ileocecal junction. After serving meals of different proportions to subjects, the percent starch which could be recovered was 2.3 to 20.1% (mean of 9.3%) for smaller meals, and 2.2 to 10.9% (mean of 6.0%) for uje larger meals. The conclusion drawn from this work was that 2 to 20% of the dietary starch escaped absorption in the small intestine.

Starch digestibility may also be dependent on cooking time, preparation of dry beans or the type of starch itself. Accordingly, 5 to 15% of bean starch can remain indigestible even after prolonged cooking (Hellendoorn, 1969). Faki and Bhavanishangar (1983), using in-vitro and in-vivo studies, showed that apart from oligosaccharides uhe starch and hemicelluloses of chickpea, cow pea and horse gram contributed substantially to the total flatulent effect; roasting or boiling were ineffective. In an effort to remove the oligosaccharides and hemicelluloses by preliminary water soaking and sieving followed

by precipitation of protein to obtain a product essentially flatus free for human consumption, the bean was rendered non-nutritive.

Methods for Detection

Flatus is the explusion of gasses that have been generated in the alimentary canal. The methods used to evaluate the flatulence causing factor(s) vary considerably. Early methods used consisted of measuring the hydrogen content of expired air (Calloway and Murphy, 1968). There was a sharp rise in the hydrogen concentration of the breath followed by an increase in rectal carbon dioxide and hydrogen with the consumption of a gas forming food. Hydrogen was a by-product exclusively of the mircro flora. These findings set the ground work for newer and more accurate methods of measuring gas by hydrogen excretions in rats, collecting egested flatus in humans, and microbiological methods.

The collected hydrogen from rats is performed in a closed life support system (Gumbmann and Williams, 1971). Expired air is collected and the hydrogen content is determined by gas chromatagraphy with a thermal conductivity detector. The coefficient of variation for the measured gasses in the expired air can range anywhere from 24 to 80%. Variations can be attributed to pretest diets, duration of legume feeding, and the contribution of hydrogen and methane from feces that collect in the gas chamber.

When human flatus is measured, colostomy sacks are connected to the buttocks as collection bags (Calloway, 1968; Levitt, 1971). Problems arise in measuring the expired gas when using humans because relatively constant levels of gas excretion are hard to establish before the experimental phase can begin. This causes considerable

variation along with a large financial cost.

To provide a rapid and economical method for measuring flatus volume, workers have attempted to assay flatulence by measuring the amount of gas produced in-vitro by bacteria. The most popular method here is using an in-vitro anaerobic culture system (Steggerda, 1968; Rockland et al., 1969). Syringes or vessels filled with substrate, thioglycolate media and inoculum are intrinsic to this procedure. The bacteria inoculum could be mixed from human colonic residues, dog intestines or pure cultures of E. coli or Cl. perfrigens. The gasses generated were analyzed for CO_2, O_2, N_2, and H_2 using a gas partitioner. In all cases, more gas was produced when the substrate contained common mono- or disaccharides then when bean flour was added.

Fermentation

In anaerobic respiration the fundamental respiratory mechanism (that varies from aerobic respiration) is nitrate or sulfate as electron acceptors for inorganic molecules. The materials for bacterial respiration include carbohydrates, proteins and the other fermentable substrates which will be converted simultaneously into volatile fatty acids (VFA), lactic aicd, methane, carbon dioxide, hydrogen, hydrogen sulfide, alcohols, ammonia, and microbial cells.

Cellulose and starch. Cellulose digestion occurs by the action of cellulase and involves the production of extra-cellular enzymes by cellulolytic bacteria. The bacteria degrade cellulose to linear chains of anhydroglucose, to oligosaccharides and finally to cellobiose. The cellobiose is then split into glucose once again by

extra-cellular enzymes. Starches and dextrans are degraded by amylases to maltose and then by maltases and maltose phosphorylases to the formation of glucose-1-phosphate (Walker, 1965). The glucose from starch and cellulose will enter the Embden-Meyerhof pathway of glycolysis (Baldwin, 1963, 1965). The C_3 compounds of pyruvate or lactate are then produced.

Hemicelluloses and pectins. Hemicellulose degradation and metabolism is not fully understood (Dehority, 1968). It is known that a nonspecific catalysis occurs at the β-1,4-xylosidic linkage in the hemicelluloses to produce xylose, xylooligosaccharides and xylobiose. Xylose is a pentose formed from xylobiose and its utilization appears to involve hexose synthesis (Pazur et al., 1958) via transketolase and transaldolase reactions. Final products are fructose-6-phosphate and triose phosphate which are sent to the glycolytic pathway for C_3 production.

Pectin is degraded by pectinesterase which hydrolyzes the 1,4-glycosidic linkages of pectic substances to form galacturonic acid. The subsequent fermentation of galacturonic acid involves the formation of pentoses which follows the pathway for hemicellulose.

Protein. Formation of VFA and gas from protein occurs when peptides and amino acids are used for energy and for biosynthetic processes. Amino acids arising from the hydrolysis of proteins may be incorporated into bacterial cells or deaminated to form ammonia, carbon dioxide, and VFA or decarboxylated to give ammonia and CO_2 (McDonald, 1948). The pathways of fermentation of amino acids by anaerobic organisms are numerous. One example is for alanine and glycine (or their deamination products) which serve as electron donors

and receptors. Another example is the formation of butyrate from glutamate via pyruvate (Barker, 1971).

Gas formation occurs from pyruvate, acetate, propionate, and butyrate production. In the case of the genus Clostridium, the transfering of electrons to protons will liberate a molecule of hydrogen. Other bacteria can transfer electrons to CO_2 and produce formate.

STUDY ONE: EVALUATION OF GENETIC VARIABILITY OF THE LOW MOLECULAR WEIGHT SUGARS IN DRY BEANS FROM A 8-PARENT DIALLEL CROSS

INTRODUCTION

Flatulence or the prouction of intestinal gas from eating cooked dry beans is considered to be one of the most important factors limiting the consumption of dry beans (Phaseolus vulgaris L.) in developed countries of the world. Hence, improvement of the image of dry beans contributing to the nutrition and health rather then social disruption due to the connotation of beans as a "poor man's diet" is necessary to enhance consumer acceptability of dry beans in the United States. Flatulence from eating cooked seeds is not peculiar to dry beans but occurs when grain of other food legumes and cereals are consumed. However, the magnitude of the intestinal discomfort appears to be most accute after eating cooked grains of P. vulgaris.

In the identification of the components responsible for flatulence Steggerda et al. (1966) found that when human subjects consumed various fractions of soybean meal [Glycine max (L.) Merr.] meal the low molecular weight carbohydrate fraction was especially potent in gas production compared to the fat, protein, and complex polysaccharide fractions. The low molecular weight fraction of soybeans contains the galactose family of oligosaccharides namely raffinose, stachyose and verbascose. Raffinose is a trisaccharide and contains a galactose, glucose and a fructose molecule with the galactose moiety having a 1-6 bond. Stachyose, a tetrasaccharide, is made up of two adjacent galactose units, one glucose and one fructose molecule with a 1-6 bond. Verbascose, a pentasaccharide, is made up of three adjacent galactose units, one glucose and one fructose

molecule joined by a 1-6 bond.

Calloway (1966), fermented stachyose with colonic bacteria and found that the bacteria could hydrolyze the 1-6 galactosyl moiety of stachyose. The enzyme responsible for this cleavage was 1-6 galactosidase which has not been found in humans (Gitzelmann & Ajurricchio, 1965). This finding by Calloway (1966) led to the speculation that sugars of the raffinose family (raffinose, stachyose and verbascose) may cause much of the intestinal discomfort associated with eating dry beans. These sugars are not absorbed in the mammalian small intestine dueto the presence of the 1-6 bond. Hence, the galactose oligosaccharides are fermented by bacteria in the lower gastrointestinal (GI) tract where they are metabolized and cleaved into 2 and 3 carbon fragments with the production of gas.

Cristofaro et al. (1973) reported diets containing stachyose and verbacose exhibited the highest flatus activity. Rackis et al. (1970), using an in-vitro assay, showed toasted, dehulled, and defatted soybean meal had the gas producing factors of sucrose, raffinose and stachyose and the gas-inhibiting factors of phenolic (syrigic and ferulic) acids. The lipids, proteins and water-insoluble polysaccharides of soybean meal had no gas activity. Fleming (1981), using rats, showed a significant positive correlation between hydrogen production and 1) stachyose, 2) raffinose plus stachyose, and 3) glucans and pentosans which were hydrolyzable in dilute acid.

It is feasible that the oligosacharides could be removed by technological or genetic intervention. Since heat treatment or soaking would most likely reduce the food value of the grains because of nutrient losses, removal through plant breeding would be the best

strategy for the long term.

There is relatively little information available as to the magnitude of variation in the galactose family oligosaccharide in beans. Because plant breeders need information concerning variability among strains for a trait to identify parents for use in breeding programs, the present study was undertaken.

The specific objective of this work was to evaluate the parents and progeny of an 8 parent diallel cross in beans to ascertain the magnitude of genetic variability for the low molecular weight sugars (sucrose, raffinose and stachyose) in beans.

MATERIALS AND METHODS

Genetic materials. Eight strains of dry beans adapted to the bean production areas of Michigan and differing in seed coat color, nutritional and culinary quality, growth habit and resistance to pests and environmental stresses were used as parents in the study. The strains Brasil-2, FF 16-15-1-CM-M-M, 15-R-148, A-30, Black Turtle Soup, Sanilac, San Fernando, and Nep-2 comprised a broad genetic base and were crossed in all possible combinations in the winter of 1980-1981. The 8 parents and 56 F_1 hybrids were space planted in an unreplicated plot in a nursery at East Lansing the following summer to produce F_2 seed. Parental and F_2 seed were harvested from individual plots in late September. All F_2 seed arising from a particular cross was bulked.

Field plot procedure. Seed of the 8 parents and 56 F_2 families were part of a larger experiment and were grown at the Saginaw Valley Bean and Sugar Beet Research farm near Saginaw and at East Lansing, Michigan. Seeds were precision drilled during May and June of 1982 with a tractor mounted air planter (Taylor, 1975) into two row plots guarded on each side by the cultivar "Seafarer". Rows were 4.9 m long and 50.8 cm apart. Herbicide and fertilizer applications were made per seasonal recommendations. Mature plants were harvested and threshed from a 6 m row length of each plot in mid to late September. Samples were cleaned and sized using appropriate metal screens. Samples were stored at room temperature until ground for analysis.

Preparation for soluble sugar analysis. Each sample was ground using a Cyclone Sample Mill (UDY Corp., Fort Collins CO) until about 40 g

of flour accumulated. Flour was placed in a plastic zip lock bag and stored at room temperature until analysis.

Extraction of soluble sugars. The extraction of the soluble sugars (glucose, sucrose, raffinose and stachyose) from the bean samples was essentially the procedure of Agbo (1982). A representative flour sample of 0.5 g was placed in a 50 ml polyethylene centrifuge tube and 18 ml of 80% ethanol: 20% water (v/v), (containing 10 ppm of sodium azide as a preservative) were added. A rubber stopper was fit snugly into the opening of the tube. Each sample was placed in a horizontal water bath with a shaker mechanism and kept at 70^{o}C for 2 hours (with periodic inversion of the centrifuge tube). The samples were removed and cooled to room temperature then centrifuged at 2000 RPM for 3 minutes. One ml of 10% lead acetate (w/v) was added to the centrifuge tube for precipitation of the ethanol soluble proteins (proamines) and the tubes were centrifuged again at 2000 RPM for three minutes. Finally, 1 ml of 10% oxalic acid (w/v) solution was added to the centrifuge tube for precipitation of the excess lead acetate. The sample was once again centrifuged at 2000 RPM for 3 minutes. After the third centrifugation the sample was diluted to a final volume of 20 ml. A 15 ml volume of the supernatant was decanted in a sample bottle and stored at -4^{o}C until sugar analysis.

To begin the analyses for sugars, the samples were brought to room temperature by thawing, centrifuged, and filtered using a carbohydrate sep pak C_{18} cartridge (Water's associates) which has similar properties as the separation carbohydrate column and causes non sugar compounds to be retained. The resulting extract sample was injected onto an Alltech 600 CHC carbohydrate column (300 x 4.1 mm,

Alltech Associates, Inc., Deerfield , IL) for quantification. The elution solvent used was acetonitrile:water, which was filtered. The solution contained acetonitrile:water at about 70:30 (v/v). The proportions were optimized to permit raffinose and stachyose to elute at 5 to 6 and 7 to 11 minutes, respectively. This procedure minimized band broadening. The pump rate was adjusted to allow a flow of 1.5 ml/minute. A 20 microliter sample of the extract was injected using a 50 ul microsyringe into a model U6K Waters injector. This injector allowed the sample to be put on a bypassed injection port (no pressure exists in the bypassed state). After injection of the sample into the U6K injector loop, the bypass valve on the Waters Associates pump system Model 6000A was switched to introduce the sample to the pressurized column maintained at room temperature. A refractive index detector was utilized (Waters Associates Model R401) to ascertain the quality and quantity of sugars present.

Methods of quantitation. External standard and repetitive injection techniques (Waters Associate 1980) were used to quantitate the soluble sugars. The external standard solution containing known amounts of sugars (sucrose 0.75mg/ml; raffinose 0.125 mg/ml; stachyose 0.5 mg/ml) was prepared and injected to determine retention time of each sugar. The quantity of sugar represented by each peak was determined by integrating the peak area with an electronic intergrator (Data Module Waters Associates, Milford, MA). The experimental samples were injected onto the HPLC column and quantified by comparison with the standard sugars previously quantified.

The percentage of an individual sugar extracted from a sample was calculated according to the following formula:

$$\text{mg sugar/gm sample} = \frac{\text{Amount shown on data module (mg/ml)} \times \text{original sample value (ml)}}{\text{sample weight (g)}}$$

Statistical Analysis. Data were subjected to statistical analysis appropriate to a randomized complete block design. Data for 2 samples and 2 determinations were analyzed (Figure 1). The MSTAT computer program developed by Michigan State University was used in order to permit statistical data analysis via a high speed computer. The ANOVA subprogram "factorial" was used to perform a 2 way-analysis of variance with sampling and subsampling. Significant differences were measured on the soluble sugars, sucrose, raffinose and stachyose between the 8 parents, the 56 crosses and the 8 parents with the 56 crosses. Mean squares with significant F ratios were reported with probability levels of $P < 0.05$ (*) and $P < 0.01$ (**). An overall LSD was calculated at the $P < 0.05$ and $P < 0.01$ level.

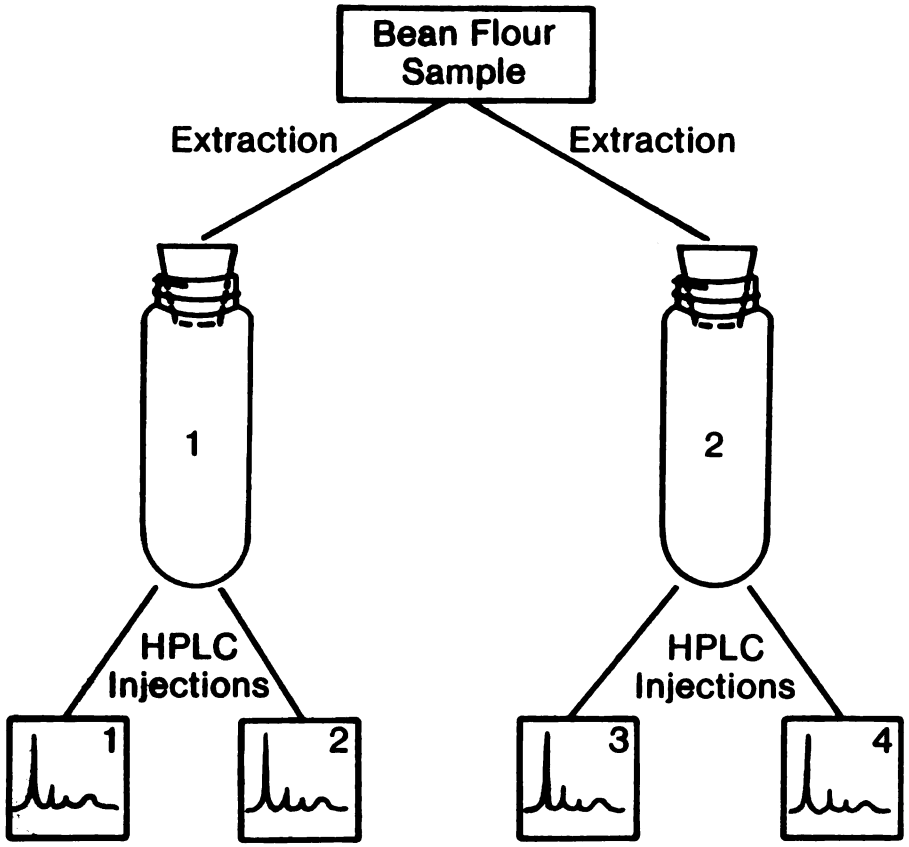

Figure 1. Flow diagram of number of extractions and injections per entry in the analysis of soluble sugars in a 8 × 8 dry bean cultivar diallel cross.

RESULTS AND DISCUSSION

Sugar oligomers of the raffinose family (raffinose and stachyose) have been implicated as the cause of flatulence associated with eating beans. Although these flatulent factors can be removed by chemical and physical means, the bean's composition is altered and may lead to a nutritionally deficient product. In the long term, it might be advantageous to remove raffinose and stachyose in dry beans by genetic intervention.

Reports concerning the feasibility of removing these sugars genetically are meager. Thus, the objective of this experiment was to ascertain the amount of variability for sucrose and the sugars of the raffinose family and assess whether or not these sugars could be altered through genetic manipulation.

Analyses of variance. The analyses were appropriate to a randomized complete block design using sampling and determinations (subsampling). The experimental design was performed on the 8 X 8 diallel cross using a two way analysis of variance. The analysis of variance was performed on the 8 parents and the 56 crosses.

The results from the Analysis of Variance (ANOVA) are in Tables 6 and 7. Results show that there were significant and highly significant differences among the parents for raffinose and stachyose respectively. Sucrose was nonsignificant as were the sampling and determination mean squares. When the 56 crosses were analyzed, differences in sucrose, raffinose and stachyose content were significant at the 1% level. The sampling and determination mean squares were nonsignificant for the progeny.

Table 6. Analysis of variances of parents for 3 sugars in a diallel cross of 8 dry bean cultivars.

		Mean Squares		
Source	df	Sucrose	Raffinose	Stachyose
Rep	1	30.13	0.72	45.28
Entry	7	175.26	11.28*	199.23**
Rep x Entry	7	85.33	1.97	8.85
Samples	16	11.50	0.70	8.92
Det.	32	8.29	0.42	1.30
Total	63			

*,** Significant @ 5 and 1% levels, respectively
df=Degrees of Freedom

Table 7. Analysis of varances of crosses for 3 sugars in a diallel cross of 8 dry bean cultivars.

		Mean Squares		
Source	df	Sucrose	Raffinose	Stachyose
Rep	1	282.42	2.58	4.65
Entry	55	703.24**	12.30**	342.42**
Rep x Entry	55	95.31	2.75	106.21
Samples	112	52.05	1.68	29.22
Det.	224	7.46	0.54	5.77
Total	447			

*,**Significant @ 5 and 1% levels, respectively
df=Degrees of Freedom

Means. The means of the entries are found in Table 8. Means of the parents varied from 17.35 to 29.26 mg/g for sucrose, 2.21 to 5.08 mg/g for raffinose, and 23.62 to 37.53 mg/g for stachyose. The means of the crosses varied from 14.88 to 58.23 mg/g for sucrose, 1.81 to 7.02 mg/g for raffinose, and from 18.29 to 52.48 mg/g for stachyose. These quantities agree with reports in the the literature for dry beans (Agbo 1982; Fleming 1981; Saulski et al., 1982; Snaw and Markakis 1976; Akpapuman and Markakis 1979; and Monte and Maga 1980).

Table 8. Means of entries over reps of 3 sugars in a diallel cross of 8 dry bean cultivars grown at E. Lansing MI in 1982.

Parents	Sucrose (mg/g)	Raffinose (mg/g)	Stachyose (mg/g)
B-2 (1)	20.59	2.21	23.62
FF (2)	22.23	2.50	26.66
15-R (3)	23.00	3.88	37.53
A-30 (4)	29.02	2.37	28.20
BTS (5)	28.43	4.27	29.67
SAN (6)	29.23	5.08	35.85
SF (7)	29.26	4.72	27.80
N-2 (8)	17.35	4.77	24.72
Crosses			
1 X 2	17.14	2.92	21.43
1 X 3	21.58	2.07	27.33
1 X 4	19.19	2.66	22.46
1 X 5	18.19	2.56	19.64
1 X 6	21.71	2.87	22.66
1 X 7	19.82	2.80	20.65
1 X 8	21.23	2.82	20.69
2 X 1	20.48	2.62	23.12
2 X 3	23.35	2.62	27.59
2 X 4	26.65	2.38	30.60
2 X 5	31.99	2.36	31.56
2 X 6	26.05	4.74	36.31
2 X 7	22.48	3.91	28.55
2 X 8	27.54	3.66	32.16
3 X 1	26.86	3.99	34.31
3 X 2	18.83	2.47	40.14
3 X 4	23.82	3.63	28.83
3 X 5	24.63	2.44	29.55
3 X 6	26.99	2.74	26.50
3 X 7	29.38	2.17	30.58
3 X 8	14.88	1.81	18.35
4 X 1	25.46	3.48	27.66
4 X 2	31.17	4.15	39.66
4 X 3	36.08	5.28	36.00
4 X 5	27.55	2.90	25.94
4 X 6	29.26	4.35	26.94
4 X 7	26.72	3.29	23.51
4 X 8	28.86	2.98	24.27

cont.

Table 8. (cont.)

	Sucrose (mg/g)	Raffinose (mg/g)	Stachyose (mg/g)
5 X 1	26.39	5.68	27.11
5 X 2	20.70	5.90	31.37
5 X 3	22.89	5.14	25.36
5 X 4	29.55	3.52	28.55
5 X 6	21.13	3.71	26.98
5 X 7	27.63	3.59	24.51
5 X 8	27.50	4.31	28.05
6 X 1	29.17	4.73	36.97
6 X 2	23.37	3.66	32.85
6 X 3	22.69	4.01	31.87
6 X 4	28.38	4.36	36.63
6 X 5	25.09	3.28	28.17
6 X 7	26.71	3.91	29.86
6 X 8	19.41	2.85	21.96
7 X 1	32.63	5.11	33.93
7 X 2	31.92	6.30	28.01
7 X 3	25.62	4.93	24.88
7 X 4	56.90	6.50	39.56
7 X 5	58.23	7.02	38.59
7 X 6	26.18	4.24	27.43
7 X 8	28.14	3.07	19.21
8 X 1	22.98	3.58	19.21
8 X 2	54.22	4.11	38.97
8 X 3	53.92	3.35	52.48
8 X 4	36.11	5.80	28.25
8 X 5	42.20	6.43	26.43
8 X 6	39.49	3.95	26.82
8 X 8	27.16	3.95	18.29
Mean	27.70	3.88	28.98
LSD 0.05	8.89	1.15	6.28

CONCLUSIONS

Results showed significant and highly significant differences among the parents for raffinose and stachyose, respectively. Differences in sucrose content were nonsignificant as were the sampling and determination mean squares. In the 56 crosses sucrose, raffinose and stachyose contained were significantly different at the 1% level. The sampling and determination mean squares were not significantly different.

The results observed indicate that significant differences in the quantity of flatulent sugars raffinose and stachyose and sucrose exist with the breeding lines chosen. This finding elicites further study for the possibility of selecting strains with low amounts of the flatulent sugars raffinose and stachyose.

STUDY TWO: QUANTIFICATION OF THE FLATULENCE FACTORS OF DRY BEANS

INTRODUCTION

After consumption by humans food legumes may create abdominal discomfort leading to diarrhea and flatulence or the production of intestinal gas. Food legumes are not unique in producing flatus because many foods produce significant amounts of intestinal gas after eating. Nevertheless, the largest flatus volumes have been attributed to eating grain legumes (Hickey et al., 1972).

It is generally recognized that gas volume produced after eating legume seeds is due to fermentation by bacteria present in the lower gastrointestinal (GI) tract of mammals. The components responsible for the production of intestinal gas by the colonic bacteria are from the portion of the grain that is not digested and/or absorbed in the lower half of the small intestine. It has been well documented that the galactose family of oligosaccharides namely raffinose and stachyose found in legume seeds, which are not hydrolyzed by humans, are fermented by colonic bacteria (Calloway 1966a; Gitzelmann & Ajurricchio, 1965; Cristofaro et al., 1973). Recent studies in dry beans (Phaseolus vulgaris L.) have indicated that even after the removal of the oligosaccharides, appreciable flatus was induced (Olson et al., 1975; Fleming, 1981, 1982_b; Hellendoorn, 1976, 1979; Kamat & Kutkarni, 1981). This led to the search for other flatulence producing compounds in bean seeds.

Another potential source of substrate for fermentation by colonic bacteria is dietary fiber. Analysis of feces for the presence of ingested fiber has shown the greater part of cellulose and lignin can be recovered in the stool (Southgate and Durnin, 1970; Williams and

Olmstead, 1936a; Williams and Olmstead, 1936b); whereas a majority of the pectins, gums and hemicelluloses are fermented by colonic bacteria (Holloway et al. 1980, 1983; Southgate 1979; Farrell et al. 1978).

It is feasible that normally digestible carbohydrates (such as starch) may not be completely digested. This may be due to consumption of carbohydrates which are less accessible or resistant to hydrolysis, a deficiency in the hydrolyzing capacity of the individual (inherited or temporary), the result of insufficient reaction time to digest and absorb the carbohydrate properly, or the result of a too rapid food transit (Hellendoorn 1978).

Stephen (1983), used human subjects which consumed mixtures of starch from navy beans, rice and potatoes found that the percent starch which could be recovered was 2.3 to 20.1%. Karimzadegan et al. (1979), using slope-ratio analysis of weight gain and plasma ketones of rats, found the apparent availabilities of the carbohydrates for digestion in soybean meal [Glycine max (L.) Merr.], lima bean (Phaseolus lunatus L.), and Bengal gram, were 35, 70 and 80%, respectively.

Low digestibility of seed protein has been observed among food legumes. Protein digestibilities have been been reported to vary from 44 to 84% for different strains of P. vulgaris (Jaffe, 1973; Kakade, 1974; Pusztai et al., 1975; Bressani, 1973). Low solubility and digestibility of legume proteins prevent hydration of protein molecules and protect peptidase-susceptible bonds from enzyme attack (De Muelenaere et al., 1961; Chen et al., 1962).

The specific objective of this study was to remove the digestible fractions from the seed of several dry bean strains and compare the remaining indigestible residue for gas producing potential with that

of several cereals used as controls.

There is a paucity of information available showing which components of the seed lead to the production of flatus among strains of dry beans. Moreover, more information is needed as to the extent the amount of gas each component is capable of producing. This information would be useful to plant breeding objectives aimed at reducing flatus potential of dry beans and improving their digestibility. Before a successful breeding strategy can be employed, plant breeders need an analytical procedure that is quantitative, and gives reproducable data.

Methodology also needs to be fairly rapid and simple and kept in perspective with costs. Hence, and in-vivo screening procedure using rats or humans would probably be too slow and complex for use by plant breeders. Hence, the objective of this study was to develop an in-vitro screening method to identify indigestible components of beans.

MATERIALS AND METHODS

Materials. Samples were limited to cereal and bean strains which were grown in Michigan production areas. The dry beans used were 'Sanilac', 'Seafarer', 'Fleetwood', 'Nep-2' and 'Proseed' cultivars of the navy commercial class. The 'Black Turtle Soup' bean, 'San Fernando' and the Pinto, 'UI-114' bean were also used. Cereals selected for comparison with beans were the soft white wheat cultivar 'Frankenmuth', pearled barley and oats obtained from a local market and the inbred corn, 'MSU 8452'.

Preparation of raw materials. The samples were prepared for analysis by hand removing all diseased and discolored seeds and debris such as hulls, stones, and dirt. Except for corn, the amount of sample ground in a Cyclone Sample Mill (UDY Corp., Fort Collins CO) yielded about 20 g of flour. The flour samples were placed in plastic bags and held at room temperature until analysis and from hence will be referred to as the "whole flour samples".

The corn sample was comminuted in a steel ground mill until the flour was able to pass through a No. 20 sieve. The coarse flour was then ground with a Wiley Mill (Arthur H. Thomas Co., Philaldelphia PA), using a No. 40 screen, and stored in the same manner as the other samples.

Proximate Analyses

Moisture. The AOAC method 13.004 (1965) was used for determining moisture content on triplicate samples of whole flour. A 3 g sample was weighed into a previously dried, cooled, and preweighed dish. The sample was dried for one hour in an air convection oven (Precision Scientific Co., Chicago IL) with ventilation and was maintained at

temperatures between 127° and 133°C. The dish was covered while still in the oven, removed, transferred to a desiccator, and weighed soon after reaching room temperature. The percent moisture was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{wet sample wt.} - \text{dry sample wt.}}{\text{wet sample wt.}} \times 100$$

Ash. Ash content was determined on triplicate samples by AOAC Method 13.006 (1965). Three g of the dried whole grain flour and 300 mg of the indigestible residue sample from each moisture determination were placed into a shallow ceramic ashing dish. The ashing dishes were previously ignited, cooled in a desiccator, and weighed soon after reaching room temperature. The samples were ignited at 550°C for 24 hours in a Barber-Coleman muffle furnace Model No. 293C (Thermolyne Corp.; Dubuque, Iowa). The white ash was allowed to cool in a desiccator and weighed soon after reaching room temperature. Percent ash was calculated as follows:

$$\% \text{ Ash} = \frac{\text{wt. crucible + ash} - \text{wt. crucible}}{\text{sample wt.}} \times 100$$

Protein. The protein content of triplicate samples from the whole grain flour and indigestible residue were determined according to the AACC Method 46-13 for crude protein. This is essentially a micro Kjeldahl procedure with a modification. A 1.30 ±0.05 g amount of K_2SO_4 was placed on weighing paper with 40 ± 5 mg HgO and between 10-30 mg of sample. The contents (excluding the weighing paper) were then placed into the Kjeldahl flask and 2.0 ml of H_2SO_4 were added.

The sample was digested for three hours at a vigorous boil. then cooled, and a minimum amount of water was added to dissolve the solids. The sample was transferred to the distillation apparatus. The flask was rinsed with approximately 10 ml of water and the procedure was carried out in the general AACC manner. Percent nitrogen was calculated as follows:

$$\% N = \frac{(\text{ml HCl} - \text{ml blank}) \times \text{normality} \times \text{equiv. wt. N}}{\text{sample wt. (mg)}} \times 100$$

The percentage of total protein was estimated by multiplying the percent nitrogen determined by 6.25.

Lipid. The lipid content was determined using a Soxhlet extraction procedure on whole flour samples in duplicate. A Tecator HT2 Soxtec System (Tecator AB; Hoganas, Sweden) was utilized and the procedure was similar to that of the modification of The National Swedish Laboratory for Agricultural Chemistry (SLL) and the Tecator Application Laboratory (Tecator manual pg 21).

Approximately 5 g (W_1) of undried flour was weighed into a Munktell cellulose extraction thimble, 33 x 80 mm. A small wad of glass wool was placed firmly over the sample material to prevent loss. The thimble was then placed into a Tecator HT2 Soxtec System for lipid extraction. Seventy ml of hexane were placed into a dry and preweighed (W_2) extraction cup containing boiling chips and the cup was inserted into the Soxtec HT. The sample was extracted for 45 minutes in the "Boiling" position and for 45 minutes in the "Rinsing" position. The hexane was evaporated and collected in a reservior by turning a stopcock positioned after the condensors which prevented the hexane to re-enter into the sample. The cup which contained the lipid

portion was released and dried at 100°C for 30 minutes, cooled in a desiccator and weighed (W_3). Percent fat (wet basis) was determined as follows:

$$\% \text{ Lipid} = \frac{(W3 - W2)}{W1} \times 100$$

Corrections to dry weight basis were made using the moisture content value determined by the method described earlier.

Starch determination. Starch content was measured using a slight modification the procedure described by Agbo (unpublished). The procedure consisted of a) solubilization of the sample; b) hydrolysis of the sample with amyloglucosidase enzyme to obtain glucose molecules; and c) injection of the hydrolysis product into a YSI model 27 Industrial Analyzer.

Starch had to be measured on the whole grain flour and the indigestible residue samples in slightly different ways due to the concentration of starch present. For starch analyses for the whole samples (30-50% starch) a sample between 19-21 mg was weighed and placed in a 50 ml polyethylene centrifuge tube with one ml of water. The centrifuge tube was fitted with a rubber stopper and placed in a boiling water bath to facilitate starch hydration. Upon cooling, 4.5 ml of 0.5N NaOH was added and the sample was homogenized with a Tekmar Tissue homogenizer (Tekmar Co.; Cincinnati, OH) for 2 minutes causing solubilization of the insoluble starch fraction. 4.5 ml of 0.50 ml glacial acetic acid was added for neutralization and the sample was once again homogenized. Next, 10 ml of a 0.04 M acetate buffer solution containing 2mg/ml of amyloglucosidase, (Glucoamylase, 1, 4, α-D-

glucan glucohydrolase; E.C. No. 3.2.1.3 from Rhizsopus genus mold), was added to the centrifuge tube. A few crystals of thymol (a preservative) was placed in the centrifuge tube and the sample was homogenized, adjusted to pH 4.5 with HCl, and incubated for eight hours in a shaking water bath at $55^{o}C$. After incubation, the sample was placed at $-4^{o}C$ until measured for glucose concentration. A blank was run along with the samples and contained the acid, base and enzyme solution. Starch measurement for the I.R. fraction (average 8% starch) was similar except for the following changes: Two ml of 0.50 N NaOH in place of the 4.5 ml, 2 ml of 0.50 N acetic acid in place of 4.5 ml, and 5 ml of the acetate buffer with 1 mg/ml of the amyloglucosidase buffer solution. This would cause a decrease in size of the blank and greater accuracy for these lower starch concentrations.

Upon calibration of the instrument, 25 ul of each individual hydrolyzed starch sample was injected into the machine, and within a minute a digital number appeared on the instrument's digital meter with the amount of glucose obtained after hydrolysis expressed in mg/dl. The blank was then quantitated and subtracted from the sample reading. Percentage of starch was calculated as follows:

$$\% \text{ starch} = \frac{\frac{\text{YSI Reading}}{1000}}{\text{sample weight}} \times 100 \times 0.9$$

The YSI model 27 glucose Industrial Analyzer (Yellow Springs Instrument Co., Yellow Springs, OH) measures sugars by the combination of immobilized enzyme and a linear electrochemical sensor. The instrument uses a thin film of oxidase enzyme according to the following equation:

$$\text{glucose} + O_2 \longrightarrow H_2O_2 + \text{glucoso-S-Lactone}$$

The hydrogen peroxide is measured by electro-chemical oxidation at a platinum anode and correlated to amount glucose for the digital display.

Recovery of indigestible residue (IR)

The objective of the in-vitro digestion procedure was to simulate human digestion of starch and protein and then collect the residue found indigestible. The procedure for enzymatic digestion of the flour sample combined the methods of Hellendoorn (1975) for indigestible residue and Asp et al. (1983) for dietary fiber analysis. This indigestible residue was further analyzed for the indigestible protein, starch and dietary fiber.

Digestion procedure. The digestion procedure was conducted in triplicate using the preground and defatted flour samples. The samples (dry beans, barley, oats, wheat and corn) were dried overnight at 100^{0} C in an air convection oven (Precision Scientific Co.; Chicago, IL) prior to digestion. This would place the sample results on a dry weight basis. An 850 mg sample of flour was accurately weighed, placed in a preweighed 250 ml polyethylene centrifuge bottle and 37 ml of water with a few crystals of thymol (preservative) were added. The centrifuge bottle had tin foil placed over the opening and was placed in a boiling water bath for 30 minutes for inactivation of inhibitors and hydration of the starch.

After boiling each sample was cooled to room temperature. The samples were divided into two groups, homogenized and nonhomogenzed. The homogenizing step was performed with a Tekmar Tissue homogenizer (Tekmar Co.; Cincinnati, OH) at low speed for 2 minutes. This proce-

dure virtually removed all clumps. In the nonhomogenized samples, clumps were broken apart manually with a glass rod. Then 5 ml of a 10 mg/ml pepsin solution (Sigma Chem. Co. EC 3.4.23.1) were added for the initial protein digestion. Each sample was adjusted using a pH meter to pH 1.9-2.0 using concentrated HCl and placed in a horizontal shaking water bath at 40°C for 4 hours. The sample was removed and readjusted using a pH meter to pH 6.8 using NaOH. Five ml of a 0.1M phosphate buffer (pH 6.8) containing 50 mg of pancreatin (Sigma Chem. Co., from Porcine Pancreas Grade VI) were added. The pancreatin buffer solution was filtered before the through Whatman cellulose filter paper to remove the cellular material from the pancreas, thus greatly reducing the amount of the indigestible residue blank. The sample was placed back into the 40°C water bath and shaken for an additional eight hours. A final volume of approximately 50 ml was obtained.

Each sample was removed from the bath, allowed to cool, and four volumes of 100% EtOH (60°C) were added and allowed to sit thirty minutes to insure precipitation. The sample was centrifuged at 2000 RPM until clear (2-4 minutes) and the supernatant, containing the amino acids and soluble sugars, was discarded. The residue was then dried overnight at 100°C in an air convection oven, removed, and placed in a desiccator and weighed upon reaching room temperature. The residue was removed from the centrifuge tube using a metal spatula, ground to a powder in a mortar with a pestle, reground in a Wiley Mill using a No. 40 mesh screen, and stored in an air tight container in a desiccator for later analysis.

Percent of indigestible residue (IR) was calculated as follows:

$$\% \text{ IR} = \frac{\text{wt. of flask with residue - wt. of flask}}{\text{wt. of sample}} \times 100$$

Reliability of procedure. From preliminary experiments a large amount of clumping ocurred after the initial boiling step. Since the design of the experiment was to simulate human digestion, it was necessary to ascertain the influence clumping had on the amount of the indigestible residue recovered. In this regard the two methods of homogenizing and nonhomogenizing were utilized after the boiling step.

It was necessary to determine the reliability of the enzymatic digestion to ascertain whether or not limiting factors existed in the time of digestion or the amount of enzymes added to the samples. This was accomplished by either doubling the time of the enzymatic digestion with the amount of the enzymes used held constant or doubling the amount of the enzymes used and holding the time constant. Because of the gravimetric nature of the procedure, blanks were run on the doubling of enzymes.

Fiber component analysis

The indigestible residue was measured directly for pectin, cellulose and ash-lignin concentration where as a combined value for hemicelluoses and gums were obtained by difference.

Pectin extraction. Pectin extraction was accomplished similar to that of Dever et al. (1967) and Robertson (1979). A 10-11 mg sample was placed in a 50 ml glass fritted centrifuge tube and 5 ml of 0.5 N ammonium oxalate ($(NH_4)_2C_2O_4$) was added. A glass stopper was fitted and the tube was placed in a shaker type water bath held at 90^{o} C for 24 hours. The tubes were removed and centrifuged at 1000 x g for 15

minutes. The supernatant (containing pectin) was decanted and saved. The residue was resuspended in 5 ml of 0.5 N ammonium oxalate and the extraction and centrifugation procedure was repeated. The supernatants were combined, the volume adjusted to 25 ml and labelled "pectin supernated". The pectin supernatant was stored at $-4^{o}C$ until analysis.

Pectin measurement. Pectin content was measured using the uronic acid estimation of Blumenkrantz and Asboe-Hansen (1973). A 200 ul sample containing approximately 0.5 ug to 20 ugs of uronic acid was added to 1.2 ml sulphuric tetraborate reagent (477 mg $Na_2B_4O_7$ $10H_2O$ to 100 ml concentrated H_2SO_4). The mixture was mixed via a vortex mixer and then cooled in ice-water. The tube was heated at $100^{o}C$ for five minutes and once again cooled in ice-water. Twenty ul of meta hydroxydiphenyl reagent (150 mg m. hydroxydiphenyl to 100 ml 0.5% NaOH, stored stoppered in refrigerator) was added and again vortexed. After five minutes from the time the tube reached room temperature, absorbance was determined at 520 nm on a Spectronic 70 Spectrophometer (Bausch & Lomb; Rochester, New York). A blank was constructed by replacing the sample solution with 0.5 N ammonium oxalate. A standard curve was produced (Figure 15) using galactouronic acid at concentrations of 1.5, 5, 10 and 15 ug/ml. Pectin concentration was measured as follows:

$$\% \text{ Pectin} = \frac{\frac{\text{ug galactouronic acid}}{1000}}{\text{wt of sample (mg)}} \times 0.9 \times 100$$

Hemicellulose and gum extraction. Extraction of the hemicellulose and gum from the cellulose and lignin was performed similar to that of Southgate (1969). Ten ml of 5% (v/v) H_2SO_4 was added to the residue

remaining in the centrifuge tube and the mixture was heated for 2 1/2 hours in a boiling water bath. The tube was removed from the bath and allowed to cool to room temperature. The mixture was filtered through a dried and preweighed 2.1 cm Whatman glass microfiber filter (934AH) and the filtrate, which contained the hemicelluloses and gums was discarded.

The percent hemicellulose and gum in the sample was calculated by subtracting the residual starch, protein, pectin, cellulose, lignin and ash from the original sample weight. A combined pergent hemicellulose and gum was calculated by difference as follows:

% Hemicellulose and gum = Total wt. - (starch, protein, pectin, cellulose, lignin-ash)

Cellulose extraction. The glass microfiber filter containing the cellulose, lignin and ash was removed and dried on a cover dish overnight in an air gonvection oven at 90°C. The filter was placed in a desiccator and after reaching room temperature and a combined value of cellulose, lignin and ash was galculated by reweighing.

The cellulose on the glass fiber filter was hydrolyzed for quantification similar to the procedures of Southgate (1969). The dried glass microfiber filter containing cellulose, lignin and ash was placed in a glass grucible with 2 ml of 72% H2S04, stirred with a glass rod and left for 24 hours at 4°C. The sample was then diluted rapidly with about 20 ml of water, centrifuged, made to 50 ml and labelled "cellulose supernatant".

Cellulose measurement. Cellulose was measured using the glucose phenol method of Lustinec et al. (1983). Three 0.5 ml aliquots of the eluate were pipetted into colorimetric test tubes and mixed with 1 ml

of 5% phenol (v/v). Exactly 2.5 ml of concentrated H_2SO_4 was added within 5 seconds with a dispenser in such a way that the steam of the acid was directed perpendicularly onto the middle of the solution surface. Immediately after the addition of the acid, the tube was agitated via a vortex mixer for 2 seconds. After vortexing, a color developed within 30 minutes and remained stable for several hours. The sample was read on a spectrophotometer at 480 nm. The optical density was estimated in the golorimeurig test tubes against a blank in which the eluate 72% H_2SO_4 was substituted. Glucose solutions ranging from 1 to 40 ug/1.0 ml were used for the construction of the calibration curve (Figure 16). The amount of cellulose was calculated as follows:

$$\% \text{ cellulose} = \frac{\frac{\text{ug glucose} \times 50}{1000}}{\text{wt of sample (mg)}} \times 0.9 \times 100$$

Lignin-ash measurement. Lignin plus ash was estimated by subtracting cellulose measured using phenol from a combined value for cellulose, lignin and ash.

RESULTS AND DISCUSSION

Any component which is not digested and/or absorbed through the small intestine will pass into the colon for possible fermentation by colonic bacteria. There are two sources for these materials: first, certain components such as fiber and the sugars of the raffinose family are not hydrolyzed into the monomeric molecules because of the absence of the specific enzymes. These polymers are too large and therefore are restricted from being transported across the gut lining. Secondly, ther are components that have the ability to be hydrolyzed and are not. They will not be absorbed and subsequently pass into the colon for potential fermentation. These components include undigested proteins, starch and fat.

Proximate Analyses

Tables 9 and 10 show the moisture, protein, fat, carbohydrate and ash gontent of the samples. All values (except moisture) are plaged on a dry weight basis.

Protein. The crude protein contents of beans agreed with ouher reports for Phaseolus vulgaris (Watt and Merrill, 1963; Meiners et. al; 1976a; Tobin and Carpenter, 1978) and as expected, dry beans were appreciably higher in protein than the cereal samples. Protein content of the beans varied from 19.9% ('Fleetwood') to 25.8% ('Seafarer'), respectively. Of the cereals, oats contained the highest crude protein at 16.5% and pearled barley the lowest at 9.0%.

Lipid. Lipids constituted a relatively small amount of the dry bean composition ranging from 0.97% ('UI-114') to 1.85% ('Fleetwood'). In the cereals, corn (4.7%) and oats (7.1%) had a fairly high lipid content compared to dry beans. Wheat and pearled barley contained

Table 9. Proximate composition of dry bean flour.[a]

	% dry basis						
	Moisture[b]	Protein[b]	Lipid[c]	—Carbohydrate—			Ash[b]	Total
				Starch[c]	Fiber[b]	S.S.[d,e]		
Sanilac	9.51 ±0.07	25.0 ±0.89	1.19 ±0.05	44.6 ±1.46	19.9	5.6	5.26 ±0.05	101.5
Seafarer	9.72 ±0.05	25.8 ±1.86	1.30 ±0.04	45.6 ±2.00	19.9	4.5	4.55 ±0.07	101.6
UI-114	10.10 ±0.02	21.5 ±0.69	0.97 ±0.05	48.3 ±1.01	17.8	5.2	4.00 ±0.13	97.8
Nep-2	10.54 ±0.04	25.4 ±2.91	1.27 ±0.06	42.8 ±2.04	19.2	4.7	4.67 ±0.20	98.0
San Fern	8.50 ±0.07	25.4 ±1.34	1.31 ±0.02	45.6 ±1.68	18.8	5.1	4.45 ±0.01	100.7
Fleetwood	8.00 ±0.09	19.9 ±1.34	1.85 ±0.02	49.1 ±1.68	18.2	5.8	4.17 ±0.01	99.0
Proseed	6.50 ±0.01	22.0 ±2.04	1.18 ±0.03	47.5 ±1.04	19.9	4.7	4.47 ±0.15	100.2

[a]Mean ±S.D.
[b]n=3 [c]n=2 [d]=4
[e]S.S.= Soluble sugars.

Table 10. Proximate composition of cereal flour.[a]

Flour	dry basis							
	Moisture[b]	Protein[b]	Lipid[c]	---Carbohydrate---			Ash[b]	Total
				Starch[c]	Fiber[b]	S.S.[d,e]		
Wheat	8.98 ±0.01	13.8 ±0.96	1.73 ±0.03	68.2 ±1.99	12.9	0.8	2.30 ±0.09	99.7
Barley	10.88 ±0.20	9.0 ±1.43	1.43 ±0.06	70.4 ±1.00	14.8	0.8	2.90 ±0.01	99.3
Oats	10.65 ±0.08	16.5 ±1.63	7.06 ±0.04	62.8 ±2.41	8.6	0.0	2.58 ±0.02	97.5
Corn	8.18 ±0.06	14.1 ±2.28	4.65 ±0.08	62.7 ±1.86	11.9	0.0	2.06 ±0.04	95.4

[a]Mean ±S.D.
[b]n=3, [c]n=2, [d]n=4
[e]S.S.=Soluble sugars
Wheat= Frankenmuth, corn=8452, Barley and oats were purchased from a local market.

less than 2% lipid in the flour samples. All values were consistent with that found in the literature (Deschamps, 1958; Watt and Merrill, 1979).

Carbohydrates. The carbohydrates in the dry bean samples included starch, mono-, di-, and oligosaccharides, and fiber. Dry beans contained a total of 65 to 68% carbohydrate. Starch constituted the major portion of dry bean carbohydrates ranging from 42.8% for 'Nep-2' to 49.1% for 'Fleetwood'. These values were similar to that found in the literature (Sathe and Salunkhe, 1981; Cerning-Beroard and Filiatre, 1976; Naivikul and D'Appolonia, 1978). The cereal samples contained from 70 to 85% carbohydrate which included starch, mono- and disaccharides and fiber. Starch also constituted the major portion of the cereals averaging from 12 to 20% higher than that found in the dry bean samples. Starch content for the cereal samples ranged from 62.7% for corn to 70.4% for pearled barley.

Ash. Total ash content was higher in the dry beans than in the cereal samples and ranged from 4.00% ('UI-114') to 5.26% ('Sanilac'). These values were consistent with that found by others (Fordham et al., 1975; Tobin and Carpenter, 1978; Kay, 1979) Ash content in the cereal samples ranged from 2.1% (corn) to 2.9% (pearled barley).

Soluble sugars. The total soluble sugar content of dry bean samples was higher than that found in the cereal samples. The dry bean's total soluble sugar content ranged from 4.5% for 'Seafarer' to 5.7% for 'Fleetwood'. Of the cereals corn, oats, wheat and barley-only wheat and barley had any detectable sugars and they contained less than 1% of dry weight.

Components within the soluble sugars. The soluble sugar components of

the dry bean and cereal samples can be found in Table 11. The external standards used in the HPLC analysis included glucose, sucrose, raffinose and stachyose.

Wheat and barley contained less than 1% sucrose while the dry bean samples contained 1.05 to 2.67% sucrose. The dry bean samples contained between 2.16 to 2.94% stachyose and 0.34 to 0.53% raffinose; this is in agreement with values reported by Tobin and Carpenter, 1978. As expected, no oligosaccharides were observed in the cereals.

Under normal conditions sucrose in the diet will be hydrolyzed and absorbed in the small intestine. The presence of raffinose and stachyose in the diet is a factor in the flatulence of dry beans. Due to the absence of 1-6 galactosidase in the alimentary tract, these oligosaccharides cannot be hydrolyzed (Gitzelmann & Ajurricchio, 1965) or absorbed through the intestinal wall (Cristofaro et al., 1973). Colonic bacteria have the capability to hydrolyze the 1-6 galactoside of raffinose and stachyose (Calloway, 1966). The sugars are fermented to provide energy for the bacteria and as a result produce gas and volatile acids as byproducts.

Bacteria in the colon will rapidly ferment these sugars compared to polysaccharides, since they are water soluble and since the structural composition is very small, from 3 to 4 molecules in length respectively.

The amount of raffinose and stachyose present in the uncooked bean is not always the amount consumed. Differences in cooking procedures will cause various amounts of the sugars to leach into the cooking broth. If the cooking broth is not consumed a high percentage of the sugars can be removed from the diet (Ku et al., 1976; Iyer et al., 1980; Koehler and Burke 1981).

Table 11. Soluble sugar concentration in various dry bean and cereal flours.[a]

Samples	Sucrose	Raffinose	Stachyose
	mg/100 mg[b]		
Beans			
Sanilac	2.34 ±0.68	0.34 ±0.06	2.94 ±0.07
Seafarer	1.05 ±0.39	0.52 ±0.15	2.93 ±0.66
UI-114	2.21 ±0.04	0.43 ±0.04	2.56 ±0.17
Nep-2	1.56 ±0.63	0.53 ±0.22	2.71 ±0.65
San Fern	2.49 ±0.95	0.45 ±0.35	2.16 ±0.29
Fleetwood	2.67 ±0.56	0.50 ±0.39	2.63 ±0.58
Proseed	2.00 ±0.26	0.42 ±0.19	2.78 ±0.86
Cereals			
Wheat	0.80 ±0.21	0.00 ±0.00	0.00 ±0.00
Barley	0.80 ±0.10	0.00 ±0.00	0.00 ±0.00
Oats	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Corn	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

n=4
[a]Mean ±S.D.
[b]Dry basis

Gravimetric determination of the indigestible residue

Recent studies indicate that even after the removal of raffinose and stachyose, dry beans can still induce appreciable flatus (Olson et al., 1975; Fleming, 1981, 1982_b; Hellendoorn, 1976, 1979; Kamat & Kutkarni, 1981; Murphy et al., 1972; Wagner, 1977). Other components such as indigestible protein, starch and dietary fiber may contribute to the flatulence associated with dry beans.

To establish the impact of uhese components in the production of intestinal gas, the indigestible residue from in vitro digestion was recovered. The indigestible protein, starch and dietary fiber were quantitated and then their potential as flatulent factors was estimated.

Recovery of the indigestible residue incorporated the procedures of Hellendoorn et al. (1975) and Asp et al. (1983). The emphasis of these workers was to avoid the harsh chemicals and subsequent destruction of various water soluble fibers used in the past (McCance, et al. 1953; Van Soest, 1973). The similarities of this experiment to Asp et al. and Hellendoorn et al. involved a two step enzymatic digestion using alimentary digestive enzymes. The first step removed digestible protein using pepsin. This is then followed by a second digestive enzyme pancreatin which includes amylases, proteases and lipases.

The amount of sample, quantity of enzyme and length of digestion was similar to that of Asp et al. (1983). Asp used an initial sample size of 1 gm and a initial 15 minute boiling for hydration of the starch. This was modified to a sample size of 850 mg and a boiling

time of 30 minutes due to changes in enzymatic digestion times. Asp et al. called for 100 mg of pepsin and a 60 minute digestion followed by 100 mg of pancreatin and an one hour digestion. In this experiment pepsin and pancreatin were decreased to 50 mg. The reason for this was the indigestible residue would eventually be used as a substrate for bacterial fermentation and the residue from the enzymes used could significantly interfer in interpretation of the results. The length of digestion was increased to 4 hours for pepsin and 8 hours for pancreatin. Adjustment of pH, precipation of the water soluble fiber using ethanol, and measurement of the indigestible starch and protein were similar to the procedures of Asp et al.

One significant variation from the methods of Asp et al. and Hellendoorn et al. was the centrifugation of the indigestible residues rather than filtration and ethanol and acetone rinses. The acetone rinse was to remove the lipid portion. The samples within this experiment were defatted so the acetone rinse was not necessary.

Homogenized vs nonhomogenizing. As a result of boiling the samples, clumps formed. It was felt that the presence of clumps during digestion would prevent access of the enzymes and thus decreases digestion of the digestible material. To break up these clumps the samples were homogenized using a tissue homogenizer which was evaluated as an extra step in the procedure.

Of the homogenized and nonhomogenized methods used (Table 12), the homogenized samples showed consistently lower residues (from 1.7% to 12.6%) compared to the nonhomogenized samples. The greatest difference was found among the cereals. A reduction in the total indigestible residue of the cereal samples varied from 3.2% for corn to a high of 12.6% for oats. The reduction in weight of the nonhomo-

Table 12. Indigestible residue content of dry bean and cereal flour.[a]

Sample	Nonhomogenized	Homogenized	% Reduction
	mg/100 mg[a]		
Beans			
Sanilac	36.2 ±0.7	34.5 ±0.6	1.7
Seafarer	39.3 ±0.5	35.2 ±0.5	4.1
UI-114	37.2 ±0.8	35.4 ±0.8	1.8
Nep-2	36.2 ±0.4	34.5 ±0.9	1.7
San Fern	37.1 ±0.8	35.3 ±0.4	2.7
Fleetwood	34.6 ±0.7	32.9 ±0.3	1.7
Proseed	38.0 ±0.4	36.2 ±0.5	1.8
Cereals			
Wheat	24.4 ±0.4	21.0 ±0.4	3.4
Barley	28.3 ±0.2	22.0 ±0.4	6.3
Oats	25.5 ±0.7	12.9 ±0.8	12.6
Corn	24.0 ±0.9	20.8 ±0.5	3.2

n=4
[a]Mean ±S.D.
[b]Dry basis

genized samples to homogenized samples in dry beans was less than 2% except for 'Seafarer' at 4.1% and 'San Fernando' at 2.2%.

The dry bean samples overall showed a higher indigestible residue (using the homogenized samples) than did the cereal samples. Of the dry bean samples, 'Fleetwood' had the lowest indigestible residue (32.9%) and 'Proseed' the highest (36.2%). In the cereal samples oats had the lowest residue (12.9%) and pearled barley the highest (22.0%).

As seen from these results, a decrease in the indigestible residue occurred when a tissue homogenizer was used to break up the clumps formed during boiling. A reason for the observed decrease in the residue in the homogenized samples compared to the nonhomogenized samples comes from a greater ease of penetration of the enzyme into the hydrolyzing sites. The tissue homogenizer may also have removed components (such as fiber) that were blocking the hydrolyzing sites (Acton et al., 1982; Harmuth-Hoen and Schwerdtfeger, 1979). Also a decrease in viscosity may occur because of the breakage of the fiber chains. This would allow an increase in the mobility or penetrability of the enzymes.

Adequacy of the digestion. The completeness of enzymatic digestion by pepsin and pancreatin by: 1) varying the length of time of the digestion and 2) the amount of enzyme present in the system.

Results indicated, using the navy bean 'Sanilac' with the homogenizing step, that the proposed method of a 12 hour digestion gave a value of 36.2% $\pm$0.71 for the indigestible resiude. When the time of digestion was lengthed to 24 hours the indigestible resiudue was measured at 36.0 $\pm$0.62. Thus doubling the length of digestion time decreased the weight of the indigestible residue by only 0.2% which

was in the range of the variation observed by the proposed procedure.

When the amount of enzyme present in the system was at the proposed level of 50 mg pepsin/50 mg pancreatin, and the time held constant, a value of 35.7 ±0.58 for the indigestible residue was observed. When the amont of enzyme was doubled to 100 mg pepsin/100 mg pancreatin a value of 35.4 ±0.61 was observed. Thus when the amount of enzyme was doubled a slight decrease in the indigestible residue of 0.3% occurred and was within the variation observed by the standard procedure.

Doubling the time and amount of enzyme had no significant effect upon the amount of indigestible residue. The final conditions developed in this procedure have been shown to be verifiable and reproducable in the estimation of the indigestible residue of grain flours.

Other factors not studied but mentioned in literature as affecting digestion are the physical form of the digestible components and the temperature and time of the original cooking process (Snow, 1981). The grinding of beans prior to cooking was associated with a 5 to 7 fold increase in the rate of starch digestion in-vitro (O'Dea and Wong, 1983; Wong et al., 1985). Observations such as these show the difficultly in the correlation of in-vitro to in-vivo studies. If the particle size resulting from grinding can influence enzymatic hydrolysis from 5 to 7 fold, the particle size that mastication produces may be a factor affecting the indigestibility of grains. The understanding and the ability to replicate the true form of the digesta from the chewing process in dry beans and cereals is needed for greater correlation between in-vivo and in-vitro studies. The samples used in this experiment were prepared by a cyclone mill which

produces one of smallest particle sizes available to the experimenter and can be viewed as the smallest particle size obtainable.

Protein and Starch in the IR.

The proximate composition of the indigestible residue from flour samples is given in Table 13. These values are based on 100 mg of the recovered indigestibile residue. Protein, starch and ash were determined directly while fiber was estimated as the remaining amount after the other constituents were subtracted from the indigestible residue.

Protein residue remaining after digestion for the samples varied from 16.1% for 'Seafarer' to 29.5% for 'San Fernando'. After digestion protein found in the cereal samples varied from 5.8% for pearled barley to 14.5% for corn. Starch content of the dry bean samples was from 13.7% for 'San Fernando' to 21.4 for 'Fleetwood'; while in the cereals, the values varied from 15.8% for oats to 19.0% for whole wheat.

The indigestible starch and protein found in the indigestible residue was converted to a whole weight basis so the amount of digestible protein and starch could be estimated (Table 14). Also, the percent of protein or starch found to be indigestible was determined by dividing the indigestible amount to that found in the whole sample. In the dry bean samples indigestible protein as a percent of the total was found to be from 22.0% for 'Proseed' and 'Seafarer' to as high as 40.9% for 'San Fernando'. However, in the cereal samples the indigestible protein ranged from 8.9% for oats to 21.4% for corn. The amount of starch that was indigestible for the dry bean samples ranged from 10.5% for 'San Fernando' to 15.4% for 'UI-114'. In the cereal samples, indigestible starch ranged from 3.2%

Table 13. Proximate composition of the indigestible residue from dry bean and cereal flours.[a]

	mg/100mg[b]			
Sample	Protein[c]	Starch[d]	Fiber[e]	Ash[c]
Beans				
Sanilac	17.8 ±0.06	14.8 ±1.62	57.6 ±0.92	9.8 ±0.2
Seafarer	16.1 ±0.40	18.6 ±0.46	56.8 ±1.98	8.5 ±0.1
UI-114	20.4 ±0.41	15.4 ±1.41	54.4 ±0.62	9.5 ±9.5
San Fern.	29.5 ±0.62	13.7 ±0.98	50.3 ±0.54	6.5 ±0.3
Fleetwood	16.6 ±0.51	21.4 ±0.62	55.3 ±0.52	6.7 ±0.4
Proseed	21.3 ±0.23	17.2 ±0.51	54.9 ±1.25	6.6 ±0.4
Cereals				
Wheat	8.3 ±0.24	19.0 ±0.52	61.4 ±2.09	11.3 ±0.2
Barley	5.8 ±0.28	16.9 ±0.81	67.2 ±1.58	10.1 ±0.4
Oats	11.4 ±0.26	15.8 ±0.24	66.7 ±2.51	6.1 ±0.3
Corn	14.5 ±0.29	18.8 ±0.26	57.1 ±0.54	9.6 ±0.6

[a]Mean ±S.D.
[b]Dry basis
[f]n=2, [c,e]n=3, [d]=4

Table 14. Content of protein and starch from dry bean and cereal flour samples found digestible and indigestible.[a]

Sample	Protein				Starch			
	Total	Dig	Ind	Ind as % of Total	Total	Dig	Ind	Ind as % of Total
Sanilac	25.0	18.9	6.14	24.5	44.6	39.5	5.11	11.5
Seafarer	25.8	20.1	5.66	22.0	45.6	39.1	6.55	14.3
UI-114	21.5	14.3	7.22	33.6	48.3	40.8	7.47	15.4
Nep-2	25.4	18.3	7.14	28.1	42.9	37.6	5.30	12.3
San Fern	25.4	15.0	10.40	40.9	45.6	40.7	4.83	10.5
Fleetwood	19.9	14.4	5.46	27.4	49.1	42.0	7.04	14.3
Proseed	22.0	14.3	7.71	22.0	41.3	35.1	6.22	15.0
Wheat	13.8	12.1	1.74	12.6	68.2	64.2	3.99	5.8
Barley	9.0	7.7	1.28	14.2	70.4	66.7	3.70	5.3
Oats	16.5	15.0	1.47	8.9	62.8	60.8	2.04	3.2
Corn	14.1	11.1	3.02	21.4	62.7	58.7	3.91	6.2

[a]Dry basis

for oats to 6.2% for corn.

Starch and protein have not traditionally been associated with the indigestible residue of food. There has been an emphasis on the complete removal of starch and protein in fiber analysis which is rightly so. The mistake occurs when one assumes that fiber is the indigestible portion of food by man. But the difficultly in completely removing the starch and protein in various grain samples is evident with the new emphasis of dietary fiber analysis being completely enzymatic (Asp, 1983; Prosky et.al 1984).

Complete removal of starch and protein in fiber analysis has been carried out by the use of nonmammalian enzymes such as bacterial heat resistant amylases, amyloglucosidase (from bacterial sources) and proteases (Asp 1983, Prosky et. al, 1984). Asp using these enzymes found P. vulgaris (brown beans) and whole wheat still had a starch residual of 0.3% and 0.1%, and a protein residual at 5.3% and 1.5% respectively.

Dropping the pH from 1.0 to 1.2 is another technique which has aided in the hydrolysis of the protein (Hellendoorn et al. 1975); but this pH is well below that observed in the stomach. The lowering of the pH for protein hydrolysis will affect and increase starch digestion. Degration of the fiber components will occur and therefore is not typical of what would be occurring under normal physiological conditions. Those using lower than normal pH conditions were still unable to completely remove the starch and protein.

It is feasible that starch, what has in the past been thought to be totally digestible, may not be completely digested. Reasons for the lack of total digestion and absorption may be that humans do not have sufficient digestive capacity relative to the amount or type of

starch consumed. This may be due to carbohydrates which are less accessible or resistant to hydrolysis, deficiency in the hydrolyzing capacity of the individual (inherited or temporary), the result of insufficient reaction time to digest and absorb the carbohydrate properly, or the result of a too rapid food transit (Hellendoorn 1978).

The resistance of the hydrolysis of proteins during enzymatic digestion has been an area of extensive research. This is because dry beans have been recommended as an alternative protein source in the lesser developed countries. Therefore, the ability to utilize the protein within the dry bean is critical.

Low digestibility of legume proteins under physiological conditions may be due to highly organized, tightly folded, compact structures which prevent hydration of protein molecules and protect peptidase-susceptible bonds from enzyme attack. This protein conformation may give resistance to proteases of the alimentary canal (De Muelenaere et al., 1971; Chen et al., 1962). Vaintraaub et al. (1975) denatured and digested the P. vulgaris protein with both pepsin and trypsin with about 5.6% of the protein still remaining unhydrolyzable.

The presence of trypsin and/or α-amylase inhibitors or interfering components will affect the ability of starch and protein to become digested. The large quantities of starch and protein or the presence of antinutritional factors found within dry beans and cereals could lead to a short intestinal transit time. The toxic lectins and polyphenols, found in dry beans and cereals may bind to the intestinal mucosa during passage through the small intestine and inhibit absorp-

tion of normally absorbable material.

The differences in digestibility of various starches have been attributed to many factors. Higher amylose content starches have been shown to be lower in digestibility. Other factors such as degree of polymerization, microheterogeneity or botanical sources of starch may affect digestibility. The presence of non-starchy components, such as protein, lipids, cellulose, hemicellulose and galactose-containing oligosaccharides may hinder hydrolysis or block absorption of the hydrolyzed starch. The nature of the digestive enzyme in-vitro or inhibition of the enzyme by a amylase inhibitors will affect the degree of digestiblity of starch (Hellendoorn 1972, 1978; Biliaderis et al. 1981).

Vaintraaub et al. (1975) denatured and digested the P. vulgaris protein with both pepsin and trypsin and found that about 5.6% of the protein remained unhydrolyzable. Rockland (1978), using in-vitro techniques, found a median of 77% with four cooked commerical types of dry legumes, compared to 93% for a casein standard. Digestibilites of the legume were consistently 15-20% lower than that of casein.

Dietary Fiber as a flatulent factor.

Dietary fiber is comprised of the indigestible polysaccharides found in food. Upon entering the colon fiber can be fermented by microorganisms causing subsequent flatus production. The disappearance of the various fiber components in the feces vary when using balance techniques with humans. Each fiber component has shown differences in the amount fermented by bacteria. Analysis of feces for the presence of fiber has showen that only a greater part of the cellulose and lignin can be recovered in the stool; most of the hemicelluloses and soluble pectins were fermented in the large intestine

(Southgate and Durnin, 1970; Williams and Olmstead, 1936a; Williams and Olmstead, 1936b; Hellendoorn, 19786). Pectin, hemicelluoses and gums show a high rate of fermentability, with cellulose showing a lower rate. Lignin and ash were found to be nonfermentable (Table 17).

The intent of this work is to quantitate the various components of fiber in the dry bean and cereal samples. An estimation of the amount each component will contribute to the total amount of flatulence can then be calculated at a latter time.

Total Fiber in IR. Total dietary fiber was estimated as the amount remaining in the indigestible residue after substraction of the indigestible protein, starch and ash (Table 15). Converting these values to a whole weight basis (Table 9) dietary fiber content for the dry bean samples varied from 17.8% for 'UI-114' to 19.9% for 'Sanilac', 'Seafarer' and 'Proseed'. In the cereal samples (Table 10), pearled barley had the highest amount of fiber in the indigestible residue at 14.8% followed by whole wheat, corn and oats the lowest (8.6%). The fiber values for whole wheat, oats and dry beans are similar to that found in the literature (Asp, 1983; Prosky et al. 1984). A literature value for whole dry corn and pearled barley was not obtained.

Composition of the Fiber. Pectin was solublized first and the supernatant was removed for uronic acid estimation. This was done because other sugars in large quantites interfere in uronic acid estimation and uronic acid can be associated with the hemicellulose fraction (Kintner et. al, 1982). No straight forward gravimetric method could be found to separate gums from the hemicelluloses and so a combined

Table 15. Fiber component content based on the indigestible residue in dry bean and cereal flour.[a]

Sample	Pectin[b]	Hemicellulose & Gums[c]	Cellulose[b]	Lignin & Ash[c]
	mg/100 mg[e]			
Beans				
Sanilac	10.03 ±0.85	22.4 ±0.19	12.8 ±0.23	12.2 ±0.29
Seafarer	8.35 ±0.82	16.6 ±1.03	13.6 ±0.87	18.2 ±0.65
UI-114	7.18 ±1.65	16.4 ±0.32	13.7 ±0.14	13.2 ±0.32
Nep-2	8.80 ±0.43	14.1 ±1.00	12.9 ±0.98	19.6 ±0.95
San Fern	7.95 ±0.65	12.2 ±1.12	12.6 ±0.21	17.6 ±1.02
Fleetwood	9.41 ±0.54	15.7 ±0.32	12.6 ±0.65	17.6 ±0.75
Proseed	9.91 ±0.99	16.1 ±0.65	11.8 ±0.53	27.1 ±1.23
Cereals				
Wheat	4.40 ±0.43	36.7 ±0.87	8.4 ±0.65	11.9 ±0.82
Barley	1.72 ±0.22	46.8 ±0.23	3.1 ±0.10	15.6 ±0.42
Oats	1.81 ±0.12	50.2 ±0.54	3.2 ±0.20	11.5 ±0.14
Corn	3.04 ±0.43	33.4 ±1.06	14.3 ±0.98	13.9 ±0.58

[a]Mean ±S.D.
[b]n=6 [c]n=2
[e]Dry basis
[b]Pectin measured by hydroxyl phenol, cellulose by glucose phenol.
[c]Hemicellullose-gums determined by difference.

value was used. Cellulose was easily hydrolyzed from the glass fiber filter using 72% H_2SO_4 and quantitated using phenol. This estimation of glucose using phenol happens under very acidic conditions. Since the glucose from the celluose was in 72% H_2SO_4 the phenol estimation was ideal in this experiment.

The total of the pectin, hemicellulose-gum, cellulose, and ash-lignin fractions is presented in Table 15 and is based on the indigestible residue. Pectin in the dry bean samples ranged from 7.18% for 'UI-114' to 10.03% for 'Sanilac'. The cereal samples showed a lower pectin concentrations which ranged from 1.72% for barley to 4.40% for whole wheat. The cellulose content of the dry bean samples were similar and ranged from 11.8% for 'Proseed' to 13.75% for 'UI-114'. Although the cellulose content of the cereals was comparably smaller than dry beans, the range in samples was greater (3.1% for barley to 14.3% for corn). The combination lignin-ash content for dry bean samples varied from 12.2% for 'Sanilac' to 27.1% for 'Proseed'. The lignin-ash content of the cereal samples was less than for dry bean samples and ranged from 11.5% for oats to 15.6% for pearled barley. The combined hemicellulose and gum content, ranged from 12.2% for 'San Fernando' to 22.4% for 'Sanilac'. The cereal samples showed similar values ranging from 30.4% for corn to 50.2% for oats.

CONCLUSIONS

Samples of dry beans (Phaseolus vulgaris) and controls of cereals were digested in-vitro for the removal of digestible protein and starch. The remaining residue (indigestible residue) was analyzed for indigestible protein and starch, for soluble sugars and for the various fiber components.

Protein residue remaining after digestion varied from 16.1% for 'Seafarer' to 29.5% for 'San Fernando'. After digestion the protein in the cereal samples varied from 5.8% for pearled barley to 14.5% for corn. Indigestible starch content of the dry bean samples were from 13.7% for 'Seafarer' to 21.4% for 'Fleetwood'; while in the cereals, values ranged from 10.8% for corn to 19.0% for whole wheat. The dry bean samples contained between 2.18 to 2.94% stachyose and 0.34 to 0.53% raffinose. Raffinose and stachyose were not observed in the cereals.

Dietary fiber content for the dry bean samples ranged from 17.8% for 'UI-114' to 19.9% for 'Sanilac' and 'Proseed'. In the cereal samples, pearled barley had the highest dietary fiber at 14.8% and corn the lowest at 8.6%.

Pectin in the dry bean samples ranged from 7.18% for 'UI-114' to 10.03% for 'Sanilac'. Pectin content in the cereals ranged from 1.72% for barley to 4.40% for whole wheat. The cellulose content of the dry bean samples ranged from 11.8% for 'Proseed' to 13.75% for 'UI-114'. Cellulose content of the cereals ranged from 3.1% for barley to 14.3% for corn. The combination lignin-ash content for dry bean samples varied from 12.2% for 'Sanilac' to 27.1% for 'Proseed'. The lignin-ash content of the cereal samples ranged from 11.5% for oats to 15.6%

for pearled barley. The combined hemicellulose and gum content, ranged from 12.2% for 'San Fernando' to 22.4% for 'Sanilac'. The cereal samples ranged from 30.4% for corn to 50.2% for oats.

STUDY THREE: FERMENTABILITY OF DRY BEANS AND CEREALS IN-VITRO

INTRODUCTION

Any material that is not digested and absorbed in the small intestine of humans is available for microbial digestion in the colon. The fermentation process will give volatile fatty acids and gas for its by-products. If the rate of fermentation becomes great enough, the excess gas or flatus produced will be expelled. Establishing a cause and effect relationship between food components and flatulence has been long and arduous. Nevertheless, work has implicated particular carbohydrates and proteins as primary flatus producing compounds.

The first suggestion that carbohydrates in dry beans may be partly responsible for flatus production was made by Anderson (1924). He demonstrated that the gasses evolved in anaerobic fermentation of various carbohydrate containing media from beans were in the form of high percentages of carbon dioxide and hydrogen and low percentages of nitrogen and oxygen.

Steggerda et al. (1966) found that when human subjects consumed various fractions of soybean [Gycine max (L.) Merr.] meal the low molecular weight carbohydrate fractions were especially potent in gas production compared with fat, protein, and complex polysaccharide fractions. Calloway (1966) subjected stachyose to fermentation by colonic bacteria and found the bacteria had the capability to hydrolyze the 1-6 galactoside residue from stachyose. The bacterial enzyme resposible for the cleavage was 1-6 galactosidase and is not present in humans (Gitzelmann & Ajurricchio, 1965). This led to the speculation that sugars of the raffinose family namely raffinose,

stachyose and verbascose may be responsible for much of the intestinal discomfort associated with eating beans.

Others (Murphy et al., 1972; Wagner, 1977) found, from measuring the amount of rectal gas produced from a bean diet in rat feeding studies, the amount of raffinose and stachyose present could not account for a majority of the gas produced.

Current research (Prynne and Southgate, 1979 Holloway et al., 1980, 1983; Hellendoorn 1978) has shown a large range for the apparent digestibility of total fiber and fiber components. Digestibilities ranged from 47 to 95% in human feces. Farrell et al. (1978) fed human subjects neutral detergent fiber and observed a complete disappearance of hemicellulose from diets with increased transit time; while 80% and 55% of the neutral detergent fiber in the diet disappeared from low and high fiber diets respectively.

Low digestibility of legume starches and proteins have also been observed (Hellendoorn 1978; Stephen, 1983; Jaffe, 1973; Kakade, 1974; Pusztai et al., 1975). But the exact fate of these components in the colon up to now has been unclear.

Flatus production associated with eating cooked grains is not peculiar to the food legumes but also occurs when grains of cereals are consumed. However, the cereals are not associated with the high flatulence in humans as found when dry beans (Phaseolus vulgaris L.) are consumed. It would be of interest to determine the amounts of gas that would theoretically be produced from various indigestible components of bean seeds and compare these with actual gas evolved from in-vitro digestion of the indgestible residue.

Hence, the present study was undertaken. Specific objectives

were to determine the theoretical and actual gas produced from dry bean seed components and compare these with cereals species used as controls. Information of this nature would be valuable to nutritionists and bean breeders for developing breeding strategy to improve the digestibility of dry beans.

MATERIAL AND METHODS

The ability of colonic bacteria to ferment analytical grade sugars and fibers and the indigestible residue was measured using a technique that consisted of: a) propagating pig colonic bacteria under anaerobic conditions for an inoculum; b) sterilizing and hydrating the samples in a serum bottle fitted with a rubber septum; c) placing the inoculum into the serum bottle for fermentation; and d) measuring the volume of gas evolved from the bacteria by water displacement.

Bacteria source. The original bacteria were obtained from fresh feces of pigs at the MSU pig barns, placed in a 500 ml beaker and covered with parafilm. The feces was transported back to the lab where it was immediately treated with O_2 free CO_2. The bacteria were transferred anaerobically to a 1000 ml sterilized erylenmeyer flask that was flushed with O_2 free CO_2. Ten ml of modified peptone yeast (PY) basal media (Table 16) was added per 1 g of feces. The flask was fitted with an air lock allowing the mixed bacteria culture to multiply under anaerobic conditions. The flask was placed in a shaking water bath and held at 37°C for 24 hours. At the end of 24 hours the shaker was shut off to allow undigested food particles to settle. The bacterial culture suspension was decanted through a wire screen into a 1000 ml sterilized erylenmeyer flask that was being flushed with O_2 free CO_2. Again the bacteria suspension was diluted to a 1:10 volume with the modified PY media. The bacteria-media suspension was placed back into a shaking water bath (37°C) and allowed to multiply for 24 hours. At the end of 24 hours the shaking action was stopped and the undigested food particles were allowed to settle for 1 hour. The bacterial suspension was decanted into a 1000 ml sterilized erylenmeyer flask

Table 16. Basal Medium - Modified Peptone Yeast Extract (PY)

Peptone	1.0 g
yeast extract	1.0 g
reaszurin solution	0.4 ml
salts solution	4.0 ml
distilled water	100.0 ml
viamin K-heme solution	1.0 ml
cysteine HCL-H_2O	0.05 g

Final resazurin concentration of 0.0001 (percent w/v). The Vitamin K-heme solution and the cysteine are aded after the medium is boiled, but before the media is autoclaved.

SALTS SOLUTION

$CACL_2$ (ANHYDROUS)	0.2 g
$MgSO_4 0_4 7H_2O$	0.2 g
K_2HPO_4	1.0 g
KH_2PO_4	40.2 g
$NaHCO_3$	10.0 g
Na_2HPO_4	33.9 g

Mix $CaCl_2$ and $MgSO_4$ in 300 ml distilled water until dissolved. Add 500 ml water and, while swirling, slowly add remaining salts. Continue swirling until all salts are dissolved. Add 200 ml distilled water, mix, and store at 4^0C.

cont.

Table 16. cont.

Vitamin K (Menadione/Hemin Solution (VK-H)

Prepare stock solution of menadione and hemin as follows:
Menadione stock solution; add 100 mg menadione to 20 ml 95% ethyl alcohol. Filter then sterilze.

Hemin stock solution: Dissolve 50 mg hemin in 1 ml 1 N NaOH; make to 100 ml with 10 ml distilled water. Autoclave at 112°C for 15 minutes

Add 1 ml sterilze meadione stock solution to 100 ml hemin stock solution. Use 1 ml of this VK-H solution in 100 ml medium.

From: Anaerobe Laboratory Manual, Virgina Polytechnic Institute and State University

being flushed with O_2 free CO_2. This suspension was used as the inoculum within one hour.

Sample preparation. Weighed amounts of analytical grade standards and the indigestible residues from the in-vitro digestion of the dry bean and cereal samples were placed in 100 ml serum bottles. A blank was used for each run in which the sample material was excluded. Under aseptic conditions, 10 ml H_2O (reduced by boiling then flushed with O_2 free CO_2, cysteine HCl and indicator) were added while the bottle was being flushed with O_2 free CO_2. The bottle was capped with a butyl septa and within 1 hour placed in an autoclave at 112°C for 15 minutes. The sterilized sample was removed from the autoclave and allowed to cool to room temperature before addition of inoculum.

Fermentation of sample. The serum bottles were placed in the 37°C water shaker type bath along with the inoculum sample for 2 hours. Thirty ml of the inoculum were placed into the sample serum bottle which was being vented and flushed with O_2 free CO_2. The inoculum was introduced with a 40 ml sterilized glass syringe. A 5 cm piece of latex hose with a No. 17 needle was attached to the tip of the syringe for withdrawal of the inoculum and addition to the serum bottle. After addition of the inoculum, the needle-latex hose was separated from the syringe and the latex hose ("reading port") was clamped. This port was used to measure the gas evolved. All pressure was allowed to escape from the serum bottle before removal of the vent needle. The sample was shaken and gas measurements were taken at various times.

Gas measurement. The gas produced by the bacteria in the serum bottle was measured by the displacement of water by the gas evolved (Fig. 2).

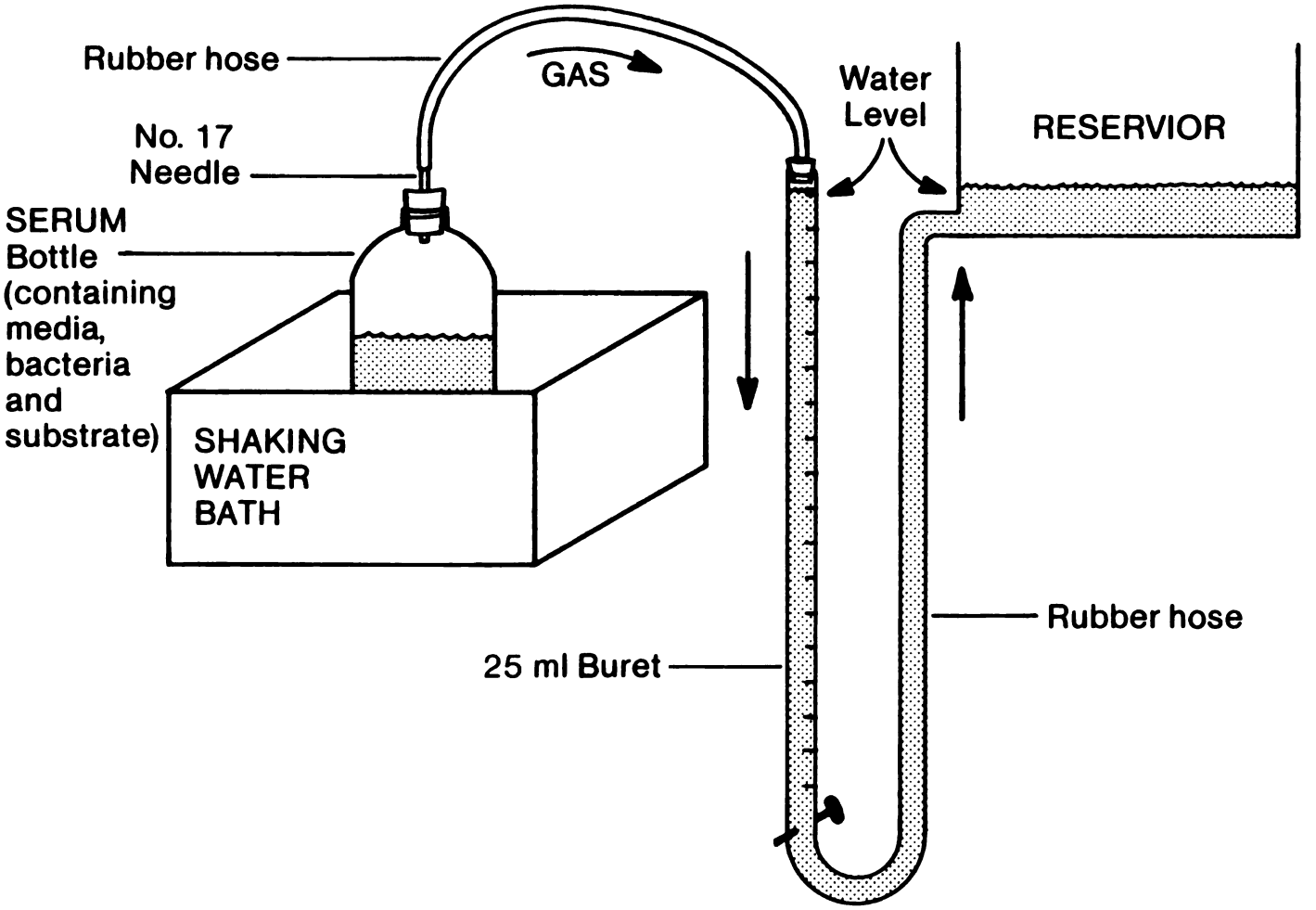

Figure 2. Apparatus for measuring bacteria gas production through anaerobic fermentation.

This was accomplished with the following apparatus: 1) a large latex hose was placed on the top of a 25 ml straight-top buret which would be eventually attached to the "reading port" of the serum bottle. 2) The stop cock of the buret was placed in the open position and a polyethylene tube was secured to the tip of the buret and passed up to a reservior bottle. The meniscus on the buret was placed at zero by adjusting the reservior. Upon measuring the produced gas from the bacteria, the large latex tube was attached to the smaller latex tube-No. 17 needle. The clamp on the needle-latex tube was removed and the gas was allowed to escape. The escaping gas displaced the water in the buret and the water displacement was recorded. The needle was again clamped until the next reading and the buret was allowed to come back to the reservoir level. The blank was also measured at each time point and the volume of gas was subtracted from each sample volume. The ml of gas measured from the serum bottles were corrected to actual ml of gas produced using a calibration curve (Fig. 3). The curve was produced by injecting known volumes of air into the latex tube with a syringe and the displacement of the water measured.

Analysis of the data. In each sample bottle, gas was measured at appropriate times which depended upon the nature of the substrate. When the rate of gas formation began to drop, the time between measurements was lengthened. The samples were no longer read after gas accumulation from the samples or standards were comparable to the blank. Those substrates which fermented rapidly were measured at two to four hour intervals and allowed to run for 24 hours. Those substrates that fermented at a slower rate were measured every 6 to 12 hours and were allowed to run for 80 hours.

Establishment of substrate to media ratio. A mono- and polysaccharide

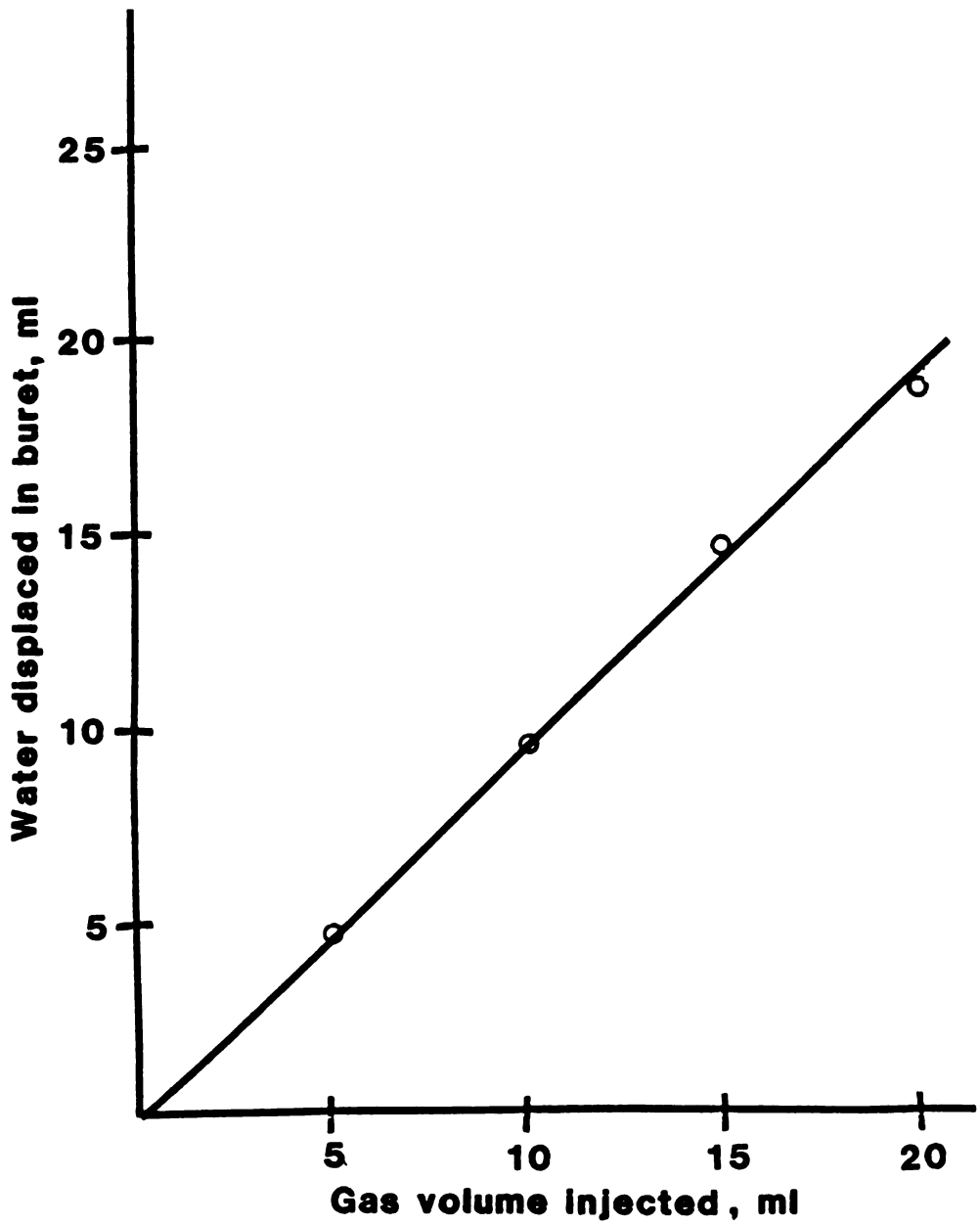

Figure 3. Calibration curve for ml of water displaced in buret vs actual ml injected.

were used to determine the proper amount of substrate needed per given volume of media to insure 100% fermentation. The carbohydrates used were glucose (Nutri. Biochem Corp.) and water soluble starch (Sigma Chem. Co., from potato). To establish the linearity of the procedure, glucose was run in duplicate in amounts of 50, 100, 150, 200 and 250 mg; starch was run in amounts of 50, 100 and 150 mg. Readings were taken hourly due to the large volume of gas that was quickly formed.

Repeatability of gas evolution during fermentation of experiments. A total of six runs were performed throughout the course of the experiment with an average of 12 samples per run. Duplicate samples of 100 mg of glucose accompanied each run to establish the reliability and repeatibility between runs.

Analytical grade standards. Protein samples included trypticase (BBL, pancreatic digest of casein), casein (Sigma Chem. Co., sodium salt) and purified phytohemaglutinin PHA-P (obtained from Don Coffey, Michigan State University E. Lansing MI, from kidney bean). The PHA was prepared as two samples: 1) autoclaving for 20 minutes at $115^{o}C$ and 2) autoclaving for 60 minutes at $115^{o}C$.

Plant fibers measured were xylan, a hemicellulose (Sigma Chem. Co., from oat spelts), pectin (Sigma Chem. Co., from citrus fruits), gum guar (Sigma Chem. Co.) and several sources of cellulose from bean hull (Testae), alfalfa meal and solka floc. The cellulose contents of the samples were quantitated by first removing the starch, protein or hemicelluloses present with a solution of 5% H_2SO_4. The procedure used was similar to the hemicellulose and gum extraction procedure except that after digestion the samples were centrifuged, washed several times with distilled H_2O, dried and ground.

Commerical corn oil was also a substrate for fermentation. Mucin (Sigma Chem. Co., Mucin II: crude from Porcine Stomach at 95 % purity) was chosen as a substrate because of its secretion in the gastrointestinal tract and its availability for fermentation in the large intestine.

Fermentation of the indigestible residue. Each indigestible residue was ground in a Wiley Mill, dried in an air convection oven at 80°C for 60 minutes and allowed to cool in a desiccator. The indigestible residues were then ready for fermentation.

Graphing results. The volume of gas accumulated in milliliters over time was pictorially described with the following information tabulated: 1) total volume of gas produced, and 2) the slope (mls of gas/hr) from the most linear portion of the graph of volume of gas produced versus time.

RESULTS AND DISCUSSION

The volume of gas that would be theoretically produced from the fermentable components in the dry bean and cereal samples (from study 2) was estimated by first measuring the quantity of evolved gas from analytical grade carbohydrates, proteins and a commerical fat. The mls of gas per given amount of the standard grade substrate were then used to estimate the volume of gas that would come from the fermentable components quantitated in the dry bean and cereal indigestible residues. Secondly, the resulting indigestible residues from in-vitro digestion were fermented to estimate the gas production potential of selected dry beans and cereals.

Anaerobic Fermentation Methodology

Development of methodology. Bacteria from pig feces were used as the inoculum for the fermentation of samples. The feces also contained components that could avoid fermentation by colonic bacteria. These components had to be removed to maintain low background interference. To remove these unwanted components the feces went through a series of dilutions and fermentations which allowed the bacteria to ferment these components (spent inoculum). Blanks were run with the samples to correct any remaining fermentable components. The volume of gas measured from the blank was subtracted from the volume of gas from the sample at each time point.

Another problem encountered in the development of the assay was the inherent production of volatile fatty acids which caused a drop in the pH. A pH of 5.9 is considered a critical point for inhibition of microorganism metabolism. The phosphate buffer concentration of the PY media was increased 10 fold to maintain a safe 6.1 pH. No other

changes were made to the modified peptone yeast (PY) media.

Establishment of substrate to media. Duplicate analyses were run throughout the experiment. Glucose and water soluble starch were used to determine the relationship between the amount of substrate and the volume of gas produced. Duplicate samples of 50, 100, 150, 200, and 250 mg for glucose and 50, 100 and 150 mg for the water soluble potato starch were used. Standards were quantified using a 40 ml volume (10 ml H_2O and 30 ml inoculum). The volume of accumulated gas produced per time can be found in Figures 4 and 5. The total volume of gas produced at the end of 24 hours was compared and the values were plotted against a given quantity of substrate (Figure 6). The correlation coefficient obtained (between 50 to 150 mg) for glucose and starch was 0.996 and 0.992, respectively. The 100 mg level was chosen as the substrate level to be used throughout the remaining experiments. At glucose concentrations of 200 and 250 mg, gas production was not proportional to the amount of substrate. This may be due to the observed drop in pH from 6.2 to 5.9.

Repeatability of gas evolution during fermentation experiments. Five experiments were performed over a two month period. Consistency throughout the experiments was monitored by running duplicate 100 mg glucose samples during each experiment. The average total amount of gas produced from the five runs of glucose was 32 ml with a standard deviation of $\pm$0.707 ml. The average slope value and standard deviation were 3.67 and $\pm$0.307 respectively (Fig 7). With this slight variation occurring between each run, the method proved to be reproducable.

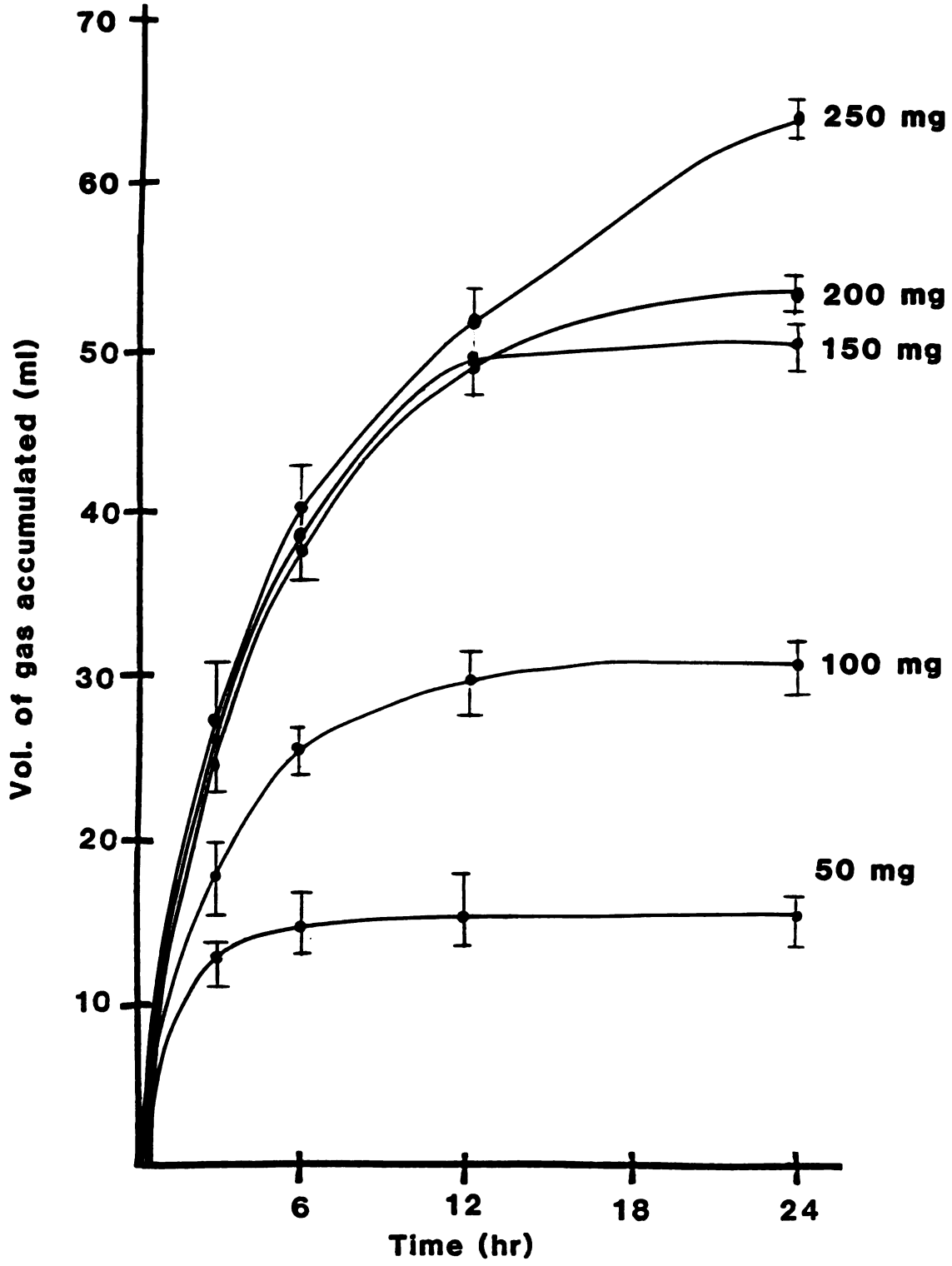

Figure 4. In-vitro gas production as related to glucose concentration.

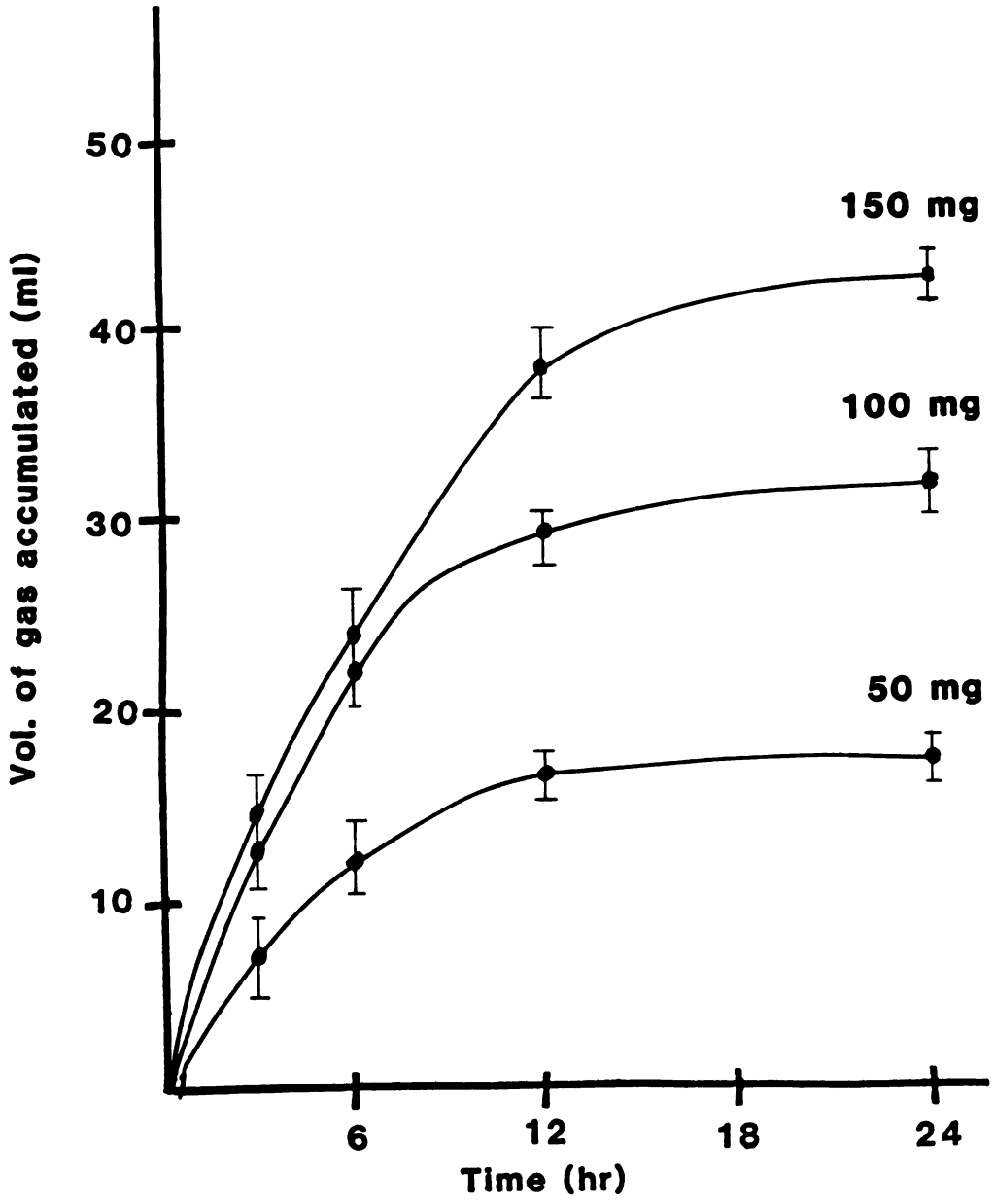

Figure 5. In-vitro gas production as related to starch concentration.

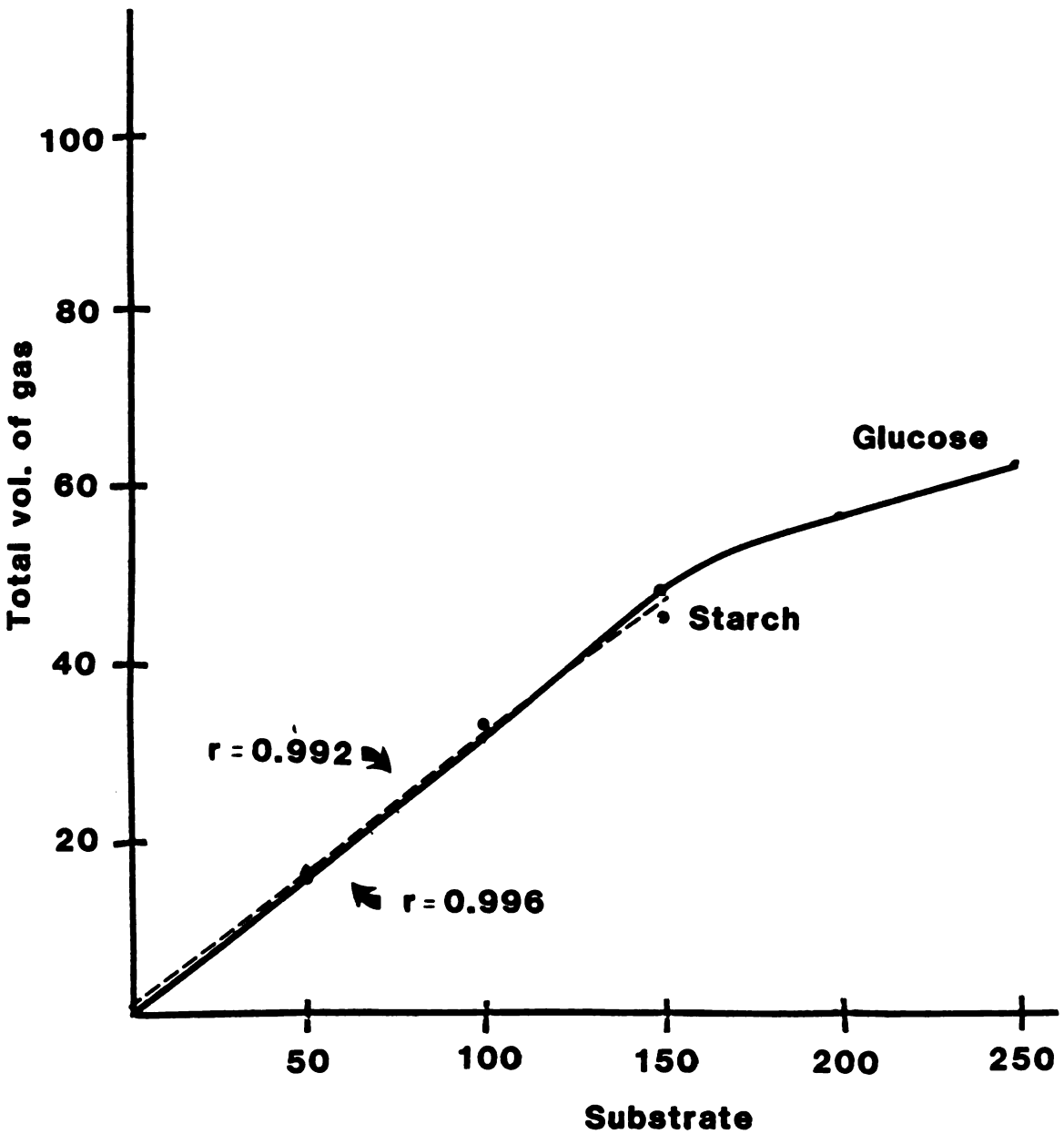

Figure 6. Standard additions of glucose and starch compared to total volume of gas produced.

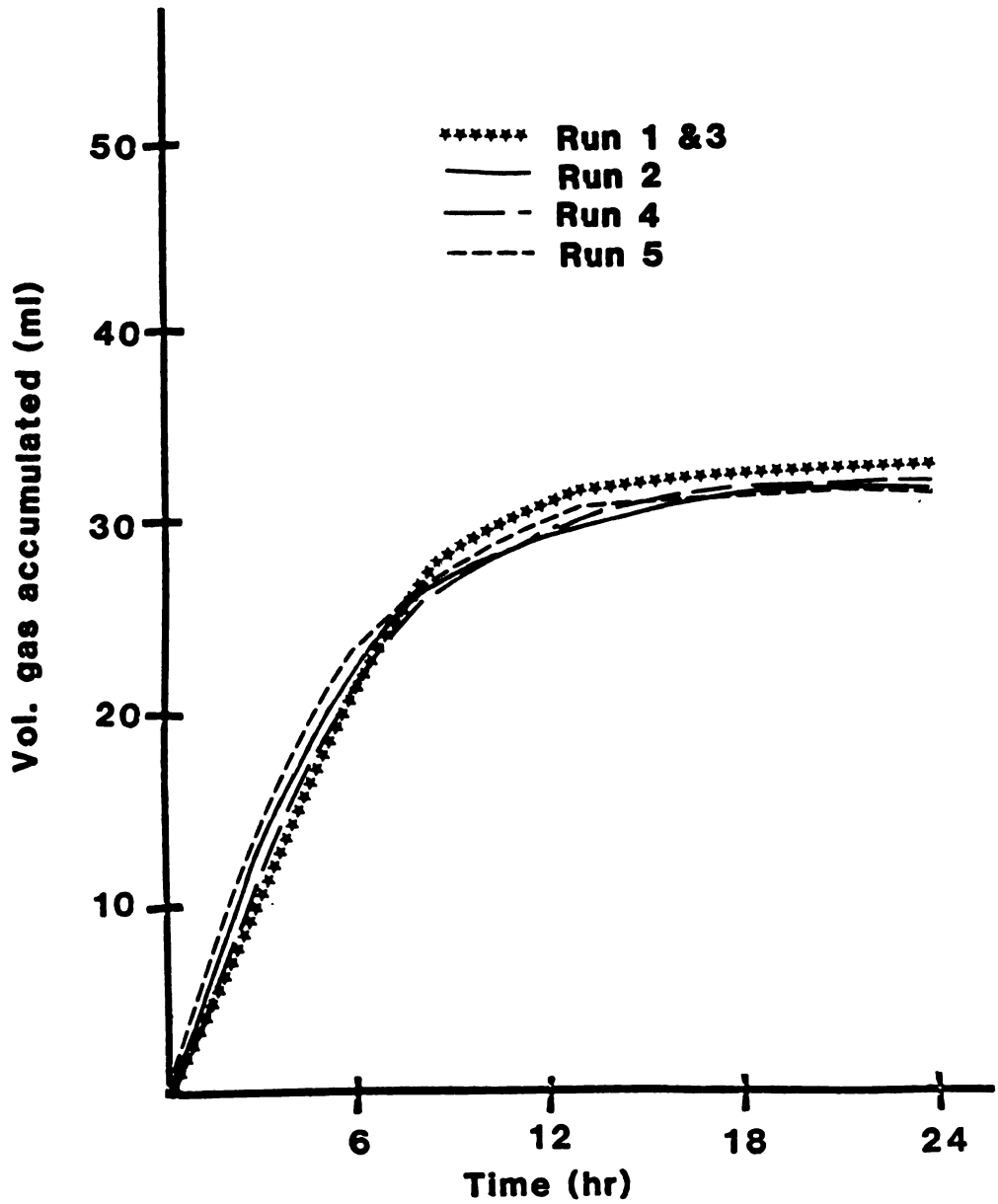

Figure 7. Comparisons of the five repetitions using 100 mg of glucose.

Anaerobic Fermentation of the Standards

Soluble sugars, starch and starch components. Comparisons of glucose, raffinose, water soluble potato starch, amylose and amylopectin are given in Figure 8. Total gas volumes produced were similar for amylose (32 ml), starch (31.4 ml), glucose (30 ml), raffinose (28 ml) and amylopectin (27 ml). When glucose was used as the standard for comparison, amylose, starch, raffinose and amylopectin produced 1.06, 0.96, 0.93, and 0.89 times as much gas respectively. As would be expected, all sugars showed different lengths of time for total digestion to occur but had similar total volumes of gas. These time differences are probably a function of the length of the monomeric chain and/or solubility. It appears bacteria preferentially favor monomeric or shorter chain polysaccharides. This is seen from the rapid fermentation of glucose and raffinose compared to the starch components amylose and amylopectin. The cleaving of the polysaccharide into its respective monomer can be considered a time consuming step and a factor in understanding the ability of components to become fermented in the colon.

The slope from the gas volume accumulated versus time was calculated from the most linear portion of the curve. This value is indicative of how rapid the substrate will ferment in the colon compared to other compounds. Also, it would be expected the greater the slope, the greater the likelihood for bacterial fermentation. The calculated slopes showed that glucose, starch, raffinose, amylose and amylopectin produced 4.37, 3.93, 2.09, 1.00 and 0.66 ml of gas per hour. Using glucose as the comparison, starch, raffinose, amylose and amylopectin had 0.90, 0.48, 0.23 and 0.15 times the rate of gas

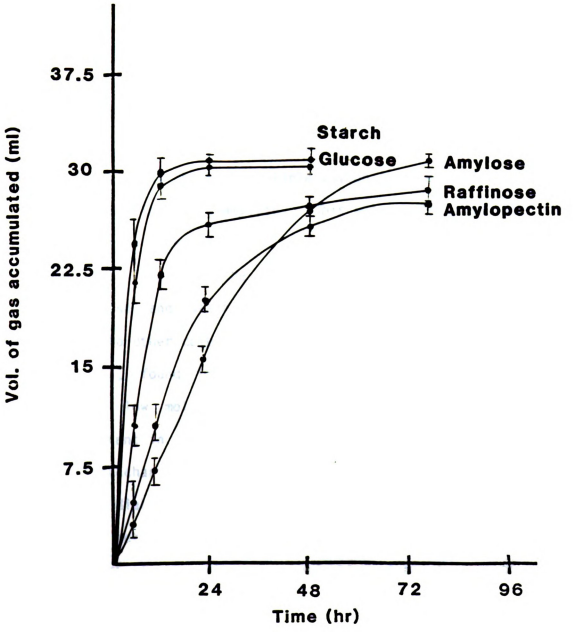

Figure 8. In-vitro fermentation of soluble sugars and starch.

production, respectively. Interestingly the slope of the polysaccharide soluble starch was found to be only slightly lower than glucose, whereas the trisaccharide raffinose showed a much slower rate of fermentation. This may indicate that bacteria can rapidly produce the α-amylase needed to hydrolyze the starch compared to the 1-6 α galatosidase needed to hydrolyze raffinose and stachyose. Both the amylose and amylopectin showed much lower rates of fermentation than starch. This is unusual since they are starch fractions. It would also be expected that the water soluble amylopectin would ferment more rapidly than the insoluble amylose. This was not the case and the reason for this is uncertain.

From the literature the rate of fermentation for the soluble sugars raffinose and stachyose have been compared to the rate of fermentation to other components. Steggerda et al. (1966), using human subjects, found that when various fractions of soybean meals were fed the low molecular weight carbohydrate fractions were especially potent in gas production compared with fat, protein, and complex polysaccharide fractions. Work done by Cristofaro et al. (1973) found diets containing stachyose and verbacose exhibited the highest flatus activity.

Protein. Figure 9 shows the gas production from fermentation of trypticase, casein, and PHA. Total volumes of gas produced were: 11.3 for trypticase, 10.5 for casein, and 6.5 ml for PHA autoclaved for 60 minutes. This was equivalent to trypticase producing 0.37, casein producing 0.35 and PHA (60 min) producing 0.22 times as much gas as glucose.

The calculated slopes showed the gas production rates of

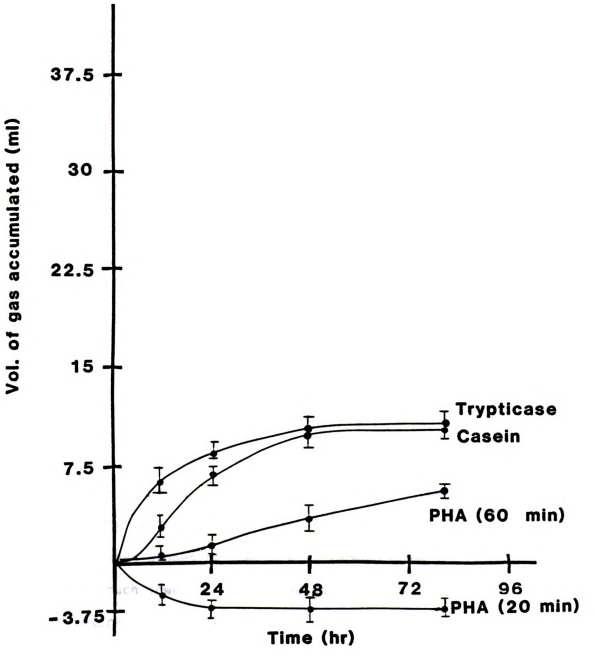

Figure 9. In-vitro fermentation of proteins.

trypticase, casein, and PHA (60 min) as 0.28, 0.26, and 0.07 ml of gas per hour, respectively. This would equate trypticase, casein and PHA (60 min) to 0.06, 0.06, and 0.02 times the rate of gas production of glucose, respectively.

As mentioned in Materials and Methods, running a blank was necessary and was subtracted out from each reading. With PHA autoclaved 20 minutes, the gas produced through fermentation had consistently lower readings than the blank causing a negative net gas accumulation. This phenomena may be due to a toxic effect from the lectin. Their known ability to hemagglutinate red blood cells may as well have an inhibitory effect on bacterial metabolism.

Trypticase and casein showed similar total volumes with trypticase having a slightly higher rate of digestion. Once again this may have resulted from trypticase being the digest of casein where the monomers or smaller peptide chains are more rapidly fermented. The PHA (60 min), assumed to be indigestable in its active or nonactive state, did show a significant fermentation rate and total gas production. It was lower in volume in comparison to the casein and trypticase samples. This may have resulted from not knowing the absolute purity or the toxicity maintained within the PHA. The total gas volume and slopes of the rate of fermentation for the protein samples were much lower than observed in the soluble sugars. This may lead to excretion of dietary protein in the feces and lower its importance as a flatulent factor.

Olson et al. (1975) and Van Stratum & Rudrum (1979) reported that the protein rich fractions from California Small White beans (CSW) and soybeans did not significantly contribute to flatulence in rats and humans.

Pectin, hemicellulose, gums and cellulose. Figure 10 shows gum, xylan and pectin had similar patterns and volumes of gas production during fermentation. Total volumes of gas produced were pectin at 30.5 ml, gum 29.5 ml and xylan 27.0 ml. This was equivalent to pectin having 1.01, gum 0.92 and xylan 0.90 times the volume of gas production then glucose. The cellulose samples (alfalfa meal and bean hull) produced a total gas volume of 15.7 and 7.5 ml, respectively. This was equivalent to alfalfa meal having 0.5 times and bean hull 0.25 times as much gas produced as observed for glucose. Solka floc had a total volume of 9.7 ml and showed an uncharacteristic sharp rise at the end of 80 hours. This indicated total digestion may not have occurred yet.

The calculated slopes showed the gas production of gum, xylan, and pectin, at 1.07, 0.99, and 0.85 ml of gas per hour, respectively. This would equate gum, xylan and pectin to having 0.24, 0.23, and 0.19 times the rate of gas production as glucose.

From the data pectin, hemicellulose and gum showed fairly identical total gas volumes and rates of production. It would be safe to assume what one of these components would do in-vivo, so would the others.

Marthinsen and Fleming (1982) measured uhe breath and flatus gasses of humans consuming high-fiber diets. They found xylan and pectin diets resulted in a production of high flatus volume and hydrogen and carbon dioxide excretion. Cellulose and corn bran generally produced breath and flatus gas excretion at levels equivalent to a fiber-free diet.

Tadesse and Eastwood (1978) reported that a hemicellulose

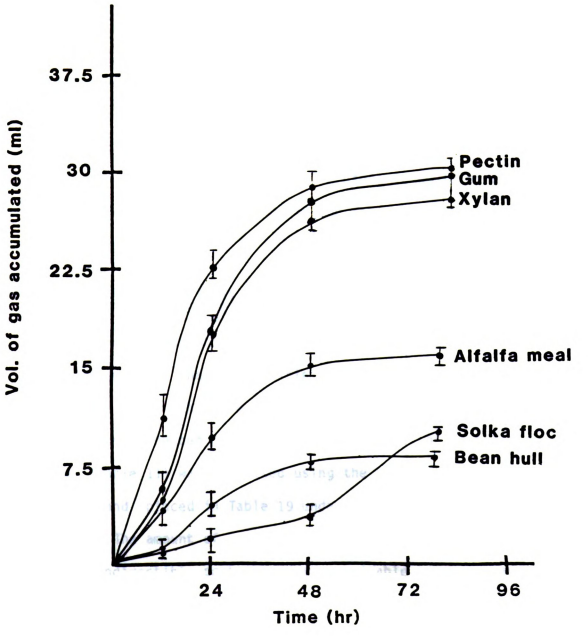

Figure 10. In-vitro fermentation of fiber components

preparation increased hydrogen production in man, but cellulose and lignin did not. Fleming (1981), using the smooth seeded field pea, found hydrogen production in the rat to be closely associated with the quantity of oligosaccharides remaining in the seed meal. However, the flatulence activity of the whole seed appeared to be due in equal parts to the indigestible oligosaccharides and components of the cell-wall fiber.

The rate of fermentation was much slower for cellulose compared to pectin, hemicellulose and gum. Therefore it would be expected a larger amount of cellulose would be in the feces than compared to the water soluble fibers.

Theoretical Amount of Gas from Fermentation of Soluble Sugars, Starch, Protein and Fiber

The volumes of gas which could theoretically be produced from the various components in the indigestible residue were estimated from the data obtained from the fermentation of standard substrates. The amount of gas produced from the total amount of raffinose and stachyose (Table 11) was estimated using the value of 30.5 ml/100 mg (Figure 8) and placed in Table 19 under the column "stachyose and raffinose". The amount of gas that could be produced from the total amount of indigestible protein and starch (Table 14) was estimated using 9.4 ml/100 mg for protein and 30.5 ml/100 mg for starch (Fig. 9 and 8) and placed in Table 19 under the column "Indigestible Protein" and under the column "Indigestible Starch".

Table 18 shows the calculated amounts of pectin, hemicellulose and gums, and cellulose that would be fermented by bacteria if present in the colon. Factors of 0.85 for pectin, hemicellulose and gums, and

Table 17. Percent of fiber and its components that are fermentable.

	1	2	3	4	5	6	7	8	Ave (%)
Fiber			55-80			70		47-82	68
Components									
Pectin	96			75				40-92	85
Hemicell.		97	100		88	96	65	40-92	86
Gum			100					40-92	85
Cellulose				40-50	42	80	69		60
Lignin						0			0

1=Holloway et al. (1983); 2=Holloway et al. (1980); 3=Farrell et al. (1978); 4=Nyman and Asp (1981); 5=Kelsay, et al. (1981); 6=Holloway (1978); 7=Slavin et al. (1981); 8=Prynne and Southgate (1978).

Table 18. Means for fiber components, amount fermentable and possible gas produced based on whole flour samples of dry beans and cereals.[a]

	mg / 100 mg						
	Pectin		Hemicellulose & Gums		Cellulose		Lignin + Ash
Sanilac	3.45	2.93[a]	7.72	6.56[a]	4.42	2.65[a]	4.21
Seafarer	2.93	2.49	5.84	4.96	4.78	2.87	6.40
UI-114	2.52	2.14	5.80	4.93	4.85	2.91	4.67
Nep-2	2.69	2.29	4.86	4.13	4.45	2.67	6.76
San Fern	2.80	2.38	4.31	3.66	4.45	2.67	6.22
Fleetwood	3.09	2.63	5.17	4.39	4.15	2.49	5.79
Proseed	3.29	2.80	5.83	4.95	4.27	2.56	9.81
Wheat	0.92	0.78	7.70	6.54	1.76	1.06	2.49
Barley	0.38	0.32	10.29	8.75	0.68	0.41	3.43
Oats	0.23	0.19	6.33	5.38	0.40	0.02	1.45
Corn	0.63	0.53	6.95	5.91	2.97	1.78	2.89

cont.

Table 18. (cont.)

	Pectin	Hemicellulose & Gums	Cellulose
	Theoretical ml of gas production[b]		
Sanilac	0.83	1.86	0.30
Seafarer	0.70	1.40	0.33
UI-114	0.61	1.40	0.34
Nep-2	0.65	1.16	0.31
San Fern	0.67	1.04	0.31
Fleetwood	0.74	1.24	0.29
Proseed	0.79	1.40	0.30
Wheat	0.22	1.85	0.12
Barley	0.09	2.47	0.04
Oats	0.06	1.52	0.00
Corn	0.15	1.67	0.20

[a]Fermentable component
[b]Estimated gas volumes per given substrate

Table 19. Content of fermentable components found in dry bean and cereal flour.

	Ind Protein	Ind Starch	Stachyose Raffinose	Fermentable Fiber	% of sample Fermentable
	mg/100 mg[a]				
Sanilac	6.14	5.11	3.5	12.1	26.8
Seafarer	5.67	6.55	3.4	10.3	25.9
UI-114	7.22	7.47	2.9	9.9	27.5
Nep-2	7.14	5.30	3.2	9.1	24.7
San Fern	10.40	4.83	2.6	7.7	25.5
Fleetwood	5.46	7.04	3.1	9.5	25.1
Proseed	7.71	6.22	3.1	10.3	27.3
Wheat	1.74	3.99	0.0	8.4	14.5
Barley	1.28	3.72	0.0	9.5	16.1
Oats	1.47	2.04	0.0	5.5	9.9
Corn	3.02	2.25	0.0	8.2	15.1

cont.

Table 19. (cont.)

	Ind Protein	Ind Starch	Stachyose Raffinose	Fermentable Fiber	% of sample Fermentable
	— Theoretical ml of gas production[b] —				
Sanilac	0.6	1.5	2.0	3.0	6.1
Seafarer	0.5	2.0	1.0	2.4	5.9
UI-114	0.7	2.3	0.8	2.4	6.2
Nep-2	0.7	1.6	0.9	2.1	5.3
San Fern	1.0	1.5	0.7	2.0	5.2
Fleetwood	0.5	2.1	0.9	4.2	7.7
Proseed	0.7	1.9	0.9	2.5	6.0
Wheat	0.2	1.2	0.0	1.7	3.6
Barley	0.1	1.1	0.0	2.6	4.0
Oats	0.1	0.6	0.0	1.6	2.4
Corn	0.3	0.7	0.0	2.1	3.5

[a]Dry basis
[b]Values for gas produced are averages from fermentations of standard substrates and are as follows: protein 9.4 ml, starch 30.5 ml, and raffinose and starchyose 28 ml per 100 mg. Fermentable fiber value comes from total found in Table 18

corresponding weight. Each calculated fermented value was in turn multiplied by appropriate values to calculate the amount of gas it could produce. Values used for quantitation of gas per given amount of pectin, gums and hemicellulose were 28.3 ml/100 mg. The value used for cellulose was 11.6 ml/100 mg (from Fig 10). The computed values were then totalled across for each sample and are given in Table 19 as "Fermentable Fiber".

From Table 19, the amount of flour found fermentable for the dry bean samples varied from 24.7 mg/100 mg for 'Nep-2' to 27.5 mg/100 mg for 'UI-114'. Of the cereal samples, oats had the lowest fermentable portion at 9.9 mg/100 mg and pearled barley the highest at 16.1 mg/100 mg. Based on this data, for every 100 mg of dry beans ingested, 5.2 to 7.7 mls of gas could be produced. While whole wheat, pearled barley, oats and corn could produce 3.6, 4.0, 2.4 and 3.0 mls of gas per every 100 mg ingested, respectively.

As can be seen from these these results, gas production from dry bean and cereal samples (Table 19) cannot be considered a function of a single component, but rather a combination of many factors. Each component-stachyose, raffinose, starch, protein, or fiber-has a different degree of fermentability and a different quantity of gas produced per given weight of sample. These differences in the amount present and the volume of gas produced is an important factor in considering the cause of flatulence. The data shown here is an in-vitro 'expectation' of the amount of indigestible components and the amount of gas that would result from their fermentation.

Anaerobic Fermentation of the Indigestible Residues

Wheat, barley, corn, oats, navy and pinto beans. Figure 11 shows the fermentation rates for whole wheat, pearled barley, oats, corn and two bean types-navy and pinto.

Total volumes of gas produced were of similar magnitude with wheat and barley having indentical fermentation rates. The volumes were as follows: navy ('Sanilac') at 30.0 ml, pinto ('UI-114') at 27.0 ml, wheat/barley 27.0 ml, oats 26.2 ml and corn 24.0 ml. This was equivalent to navy having 1.0, pinto 0.90, wheat/barley 0.9 and oats 0.87 and corn 0.80 times more gas than observed for glucose. The slopes showed the gas production from oats, wheat/barley, navy, pinto beans and corn were 1.56, 1.37, 0.60, 0.53 and 0.50 ml of gas per hour, respectively. This would be equivalent to oats, wheat/barley, navy, pinto and corn having 0.36, 0.31, 0.14, 0.12 and 0.10 times the rate of gas production of glucose, respectively.

Bean cultivars. Figure 12 compares the fermentation rates of dry bean cultivars 'Proseed', 'Sanilac', 'Seafarer', 'Fleetwood', 'Nep-2', 'San Fernando' and 'UI-114'.

Total gas volumes for the bean cultivars were as follows: 'Proseed' with 33.0 ml, 'Nep-2' at 30 ml, 'Sanilac' at 30.0 ml, 'Fleetwood' at 27.5 ml, 'UI-114' at 27.0 ml, 'San Fernando' at 25.5 ml and 'Seafarer' at 24.0 ml. This was equivalent to 'Proseed' having 1.1, 'Nep-2' 0.9, 'Sanilac' 1.0, 'Fleetwood' 0.9, 'UI-114' 0.9, 'San Fernanado' 0.85 and 'Seafarer' 0.8 times as much gas volume as observed for glucose.

The slopes showed the gas production from 'Proseed', 'Nep-2', 'Fleetwood' and 'Sanilac', 'UI-114', 'San Fernando', and 'Seafarer' were 0.74, 0.65, 0.60, 0.53, 0.49, and 0.41 ml of gas per hour,

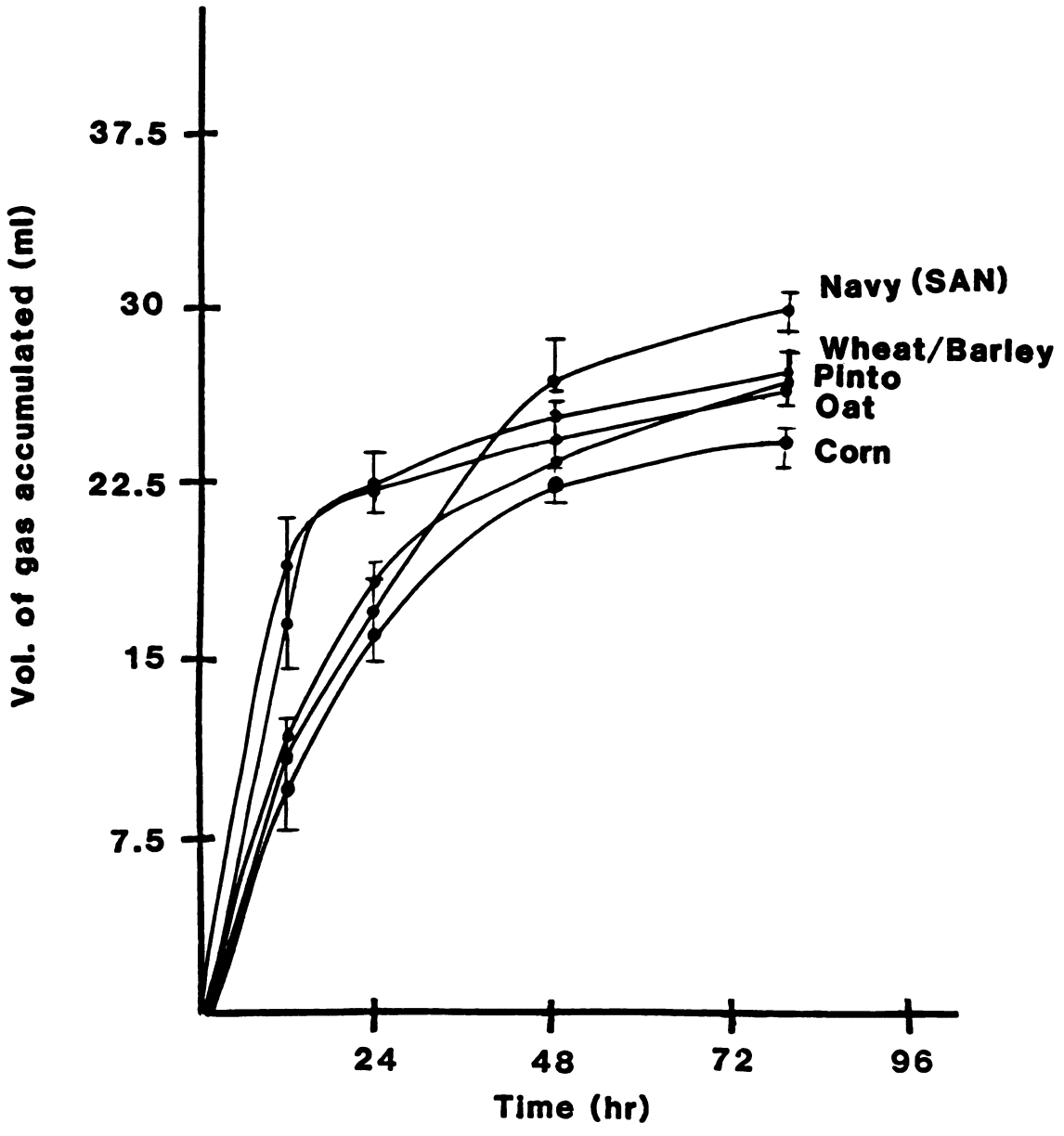

Figure 11. In-vitro fermentation of dry beans and cereal.

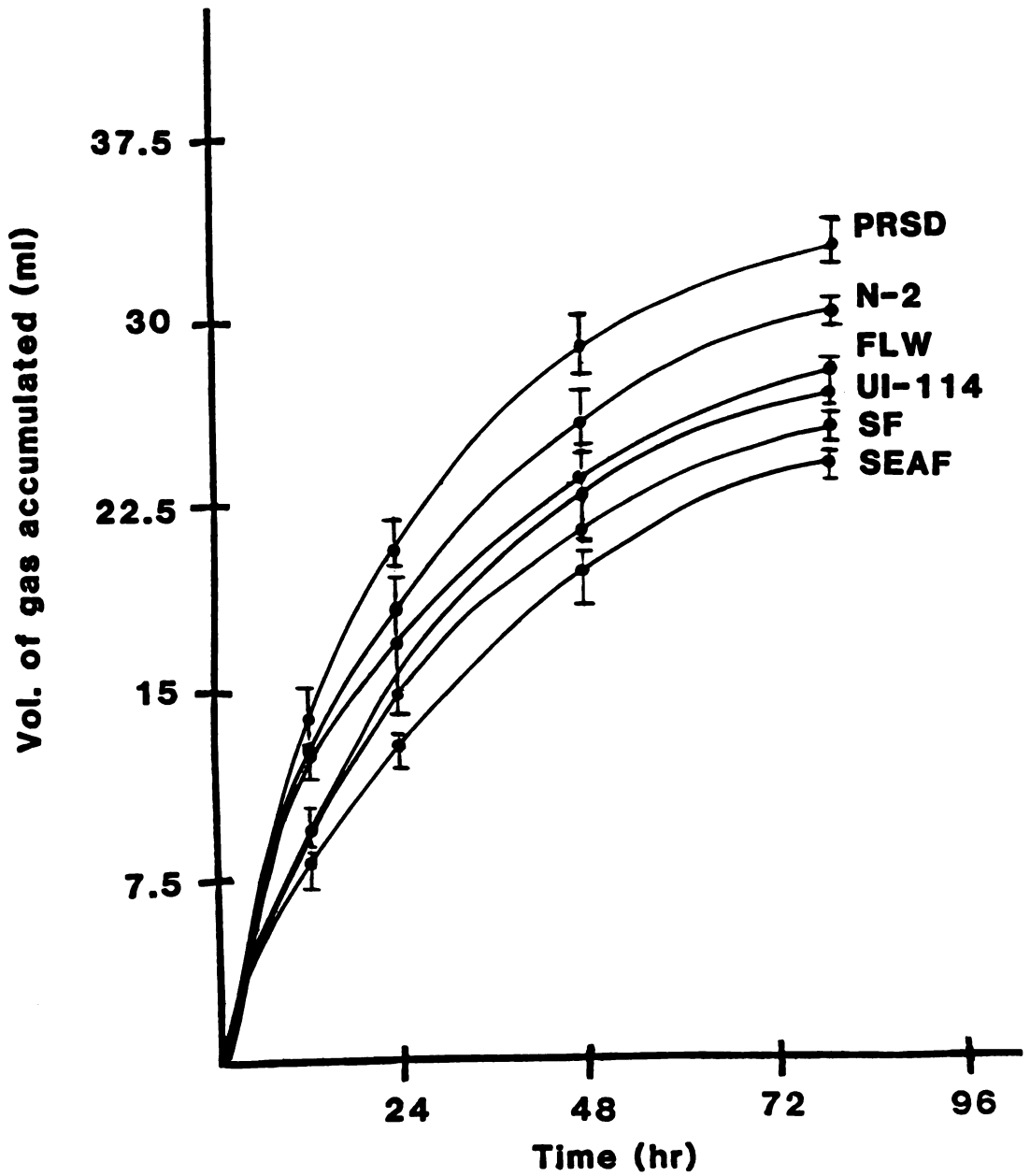

Figure 12. In-vitro fermentation of bean cultivars.

respectively. This would be equivalent to 'Proseed', 'Nep-2', 'Fleetwood' and 'Sanilac', 'UI-114', 'San Fernando' and 'Seafarer' having 0.17, 0.15, 0.14, 0.12, 0.11 and 0.09 times the rate of gas production of glucose, respectively.

Addition of lost indigestible sugars. Due to the extraction procedure used to obtain the indigestible residue, raffinose and stachyose were not included as part of uje indigestible residue; they were lost in the ethanol phase. For this reason the sugars were added back to navy bean (Fleetwood) indigestible residue in at the concentration found in the original bean sample residue.

In Figure 13 both samples produced similar total volumes of gas at 28.5 ml for 'Fleetwood' + sugars and 27.5 ml for 'Fleetwood' without the sugars. This would be expected since both samples contained 100 mg of substrate. Of greater importance was the fermentation rate of the indigestible residue with the addition of the soluble sugars. A slope of 1.10 ml of gas per hour in the sample containing the sugars and a slope of 0.60 ml of gas per hour in the sample without the sugars demonstrates that fermentation is considerably faster when oligosaccharides are present.

Fat and Mucin. Corn oil and mucin were tested for their ability to produce gas. Mucin was chosen because of its secretion in the small intestine and its expected presence in uje large intestine.

Figure 14 shows the fermentation pattern for corn oil and mucin. Total volumes of gas observed were 8.5 ml for corn oil and 14.5 ml for mucin. This was equivalent to corn oil having 0.22 and mucin 0.48 times as much gas as observed for glucose. The slopes for corn oil and mucin were 0.14 and 0.40 ml of gas per hour, respectively. This

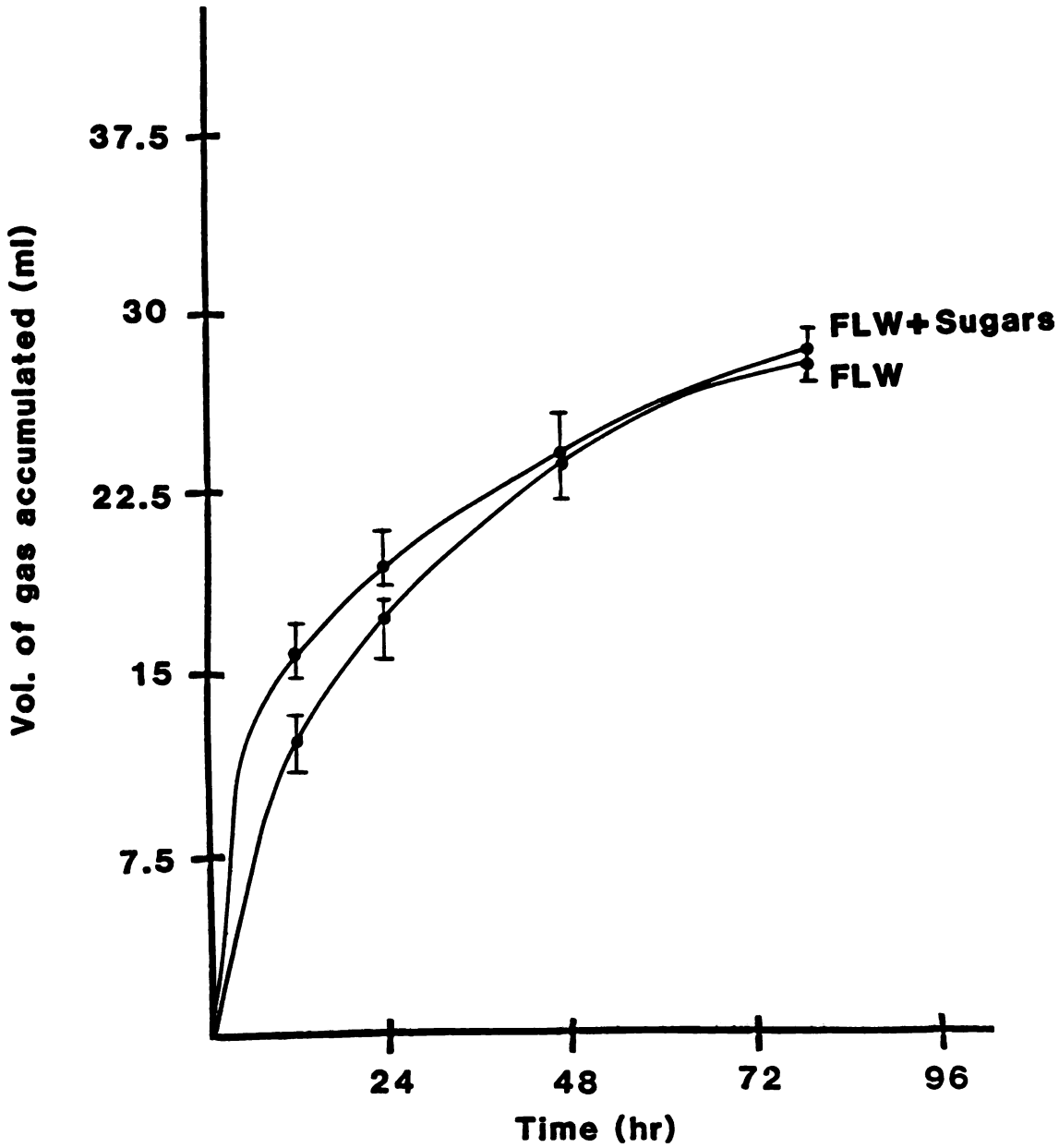

Figure 13. In-vitro fermentation showing addition of indigestible sugars.

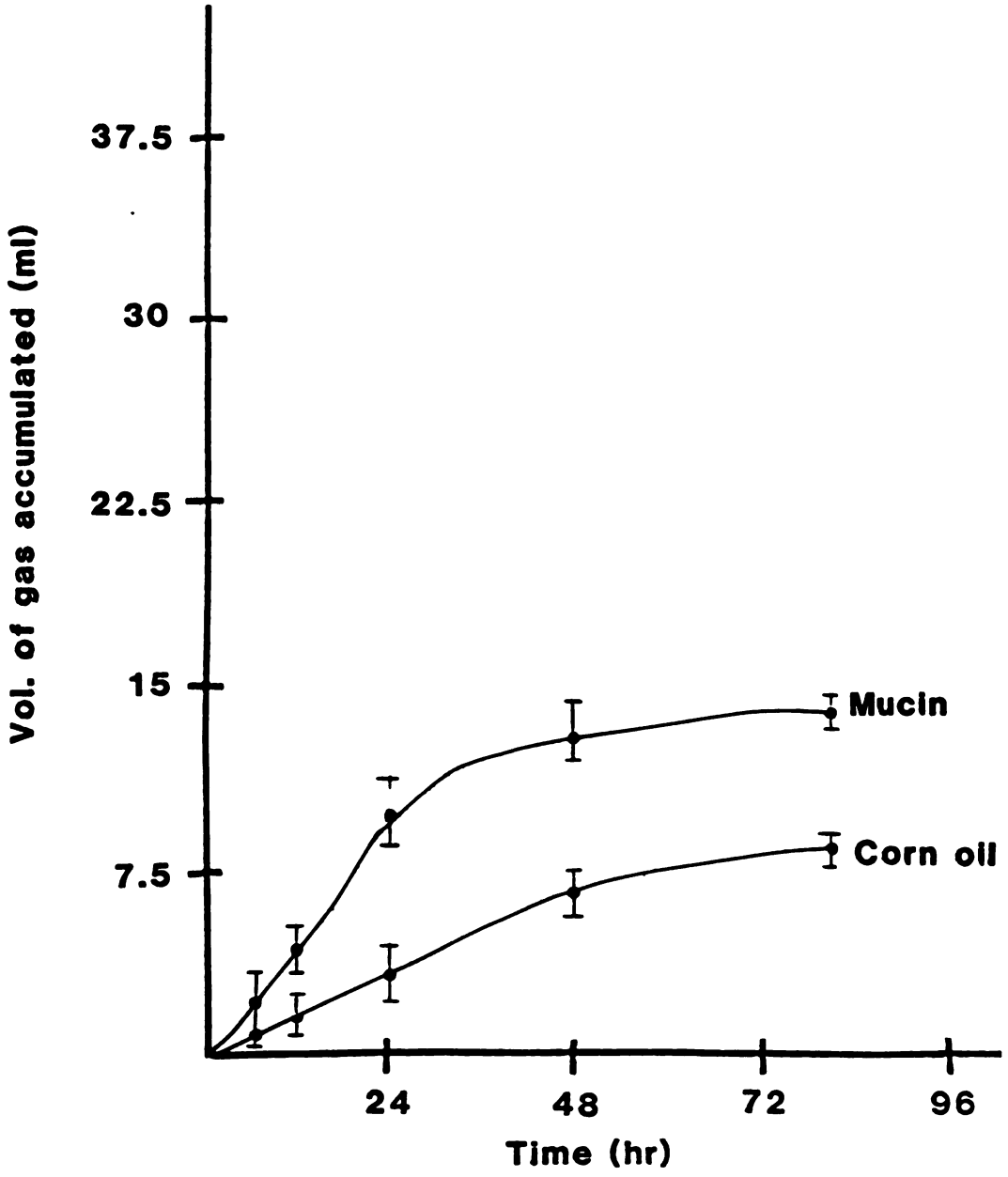

Figure 14. In-vitro fermentation of mucin and corn oil.

would be equivalent to corn oil and mucin having 0.03 and 0.09 times the rate of gas production as glucose.

Total gas volume and rate of fermentation for corn oil was similar to protein and much lower than observed in the carbohydrates. Although dry beans contain only 2 to 3% lipid, the in-vivo digestibility is not well documented. Therefore the lipid portion of dry beans could be a source of intestinal gas.

Mucin, which is an inherent part of the digestive residue, showed a fermentation rate and total gas production similar to protein but a considerably slower rate and a lower gas production than observed for the fermentation of simple and complex carbohydrates. From this observation, mucin's fermentability suggests that it too can be considered as a potential flatulent factor. The amount of mucin secreted by the GI tract may be a function of the type of material passing through it (dry beans compared to cereals); however this would need to be verified by by in-vivo studies.

Table 20 correlates the values obtained from the 'theoretical gas produced' to the values actually observed from the fermentation of the indigestible residue. The actual gas produced and the theoretical gas produced have now been calculated based on the in-vitro indigestible residue from 100 mg of whole flour. Because raffinose and stachyose were lost in the recovery of the indigestible residue, a gas value was calculated from the known amount of the sugars in 100 mg of the whole flour.

In the dry bean samples the indigestible residue from 100 mg of whole flour sample ranged from 32.9/100 mg to 36.2 mg/100 mg. The residues when fermented can produce 9.4 ml to 12.8 ml of gas. These actual gas values ranged from 1.3 to 2.1 times higher then observed

Table 20. Actual volume of gas produced by in-vitro fermentation of the indigestible residue based on 100 mg of whole flour.[a]

	Indigestible Residue mg/100 mg	Gas Produced (ml)	Raffinose Stachyose mg/100 mg	Gas Produced (ml)	Actual Gas Produced	Theoretical Gas Produced
Beans						
Sanilac	34.5	(10.3)	3.5	(1.0)	(11.3)	(6.1)
Seafarer	35.2	(8.4)	3.4	(1.0)	(9.4)	(5.9)
Pinto	35.4	(9.6)	2.9	(0.8)	(10.4)	(6.2)
Nep-2	34.5	(10.4)	3.2	(0.9)	(11.3)	(5.3)
San Fern	35.3	(9.0)	2.6	(0.7)	(9.7)	(5.2)
Fleetwood	32.9	(9.0)	3.1	(0.9)	(9.9)	(7.7)
Proseed	36.2	(11.9)	3.1	(0.9)	(12.8)	(6.0)
Cereals						
Wheat	21.0	(5.7)	0.0	(0.0)	(5.7)	(3.6)
Barley	22.0	(5.9)	0.0	(0.0)	(5.9)	(4.0)
Oats	12.0	(3.4)	0.0	(0.0)	(3.4)	(2.4)
Corn	20.8	(4.9)	0.0	(0.0)	(4.9)	(3.5)

[a] 'Theoretical gas produced' from Table 19

from the values calculated in 'Theoretical gas expectation'.

The cereals ranged from 12.9 mg/100 mg to 22.0 mg/100 mg for the indigestible residue. The residues when fermented can produce 3.4 ml to 5.9 ml of gas. These actual gas values ranged from 1.4 to 1.6 uimes higher than observed from the values calculated in 'Theoretical gas expectation'.

A reason for these higher readings may stem from the complete fermen ation of the indigestible residue; where the theoretical values were based on a percentage of the fibers that have been found fermentable from in-vivo experiments.

But this data bears out that both cereals and dry beans do produce flatulence. Overall the values of gas produced in the dry beans was approximately twice that observed in the cereals in both the actual and theoretical volumes of gas. Therefore it can be reasoned that dry beans are more nortorious in the production of flatulence.

CONCLUSION

The volume of gas that would be theoretically produced from the fermentable components in the dry bean and cereal samples was estimated by first measuring the quantity of evolved gas from analytical grade carbohydrates, proteins and a commerical fat. The mls of gas per given amount of the standard grade substrate were then used to estimate the volume of gas that would come from the fermentable components quantitated in the dry bean and cereal indigestible residues. Secondly, the resulting indigestible residues from in-vitro digestion were fermented to estimate the gas production potential of selected dry beans and cereals.

The percentages of the original sample which were fermentable ranged from 24.7 mg/100 mg for 'Nep-2' to 27.5 mg/100 mg for for 'UI-114'. Based on this data, for every 100 mg of dry beans ingested, 5.2 to 7.7 ml of gas could be produced. The indigestible residue from cereals ranged from 9.9 mg/100 mg for oats to 16.1 mg/100 mg for pearled barley. This could mean that whole wheat, pearled barley, oats and corn could produce 3.6, 4.0, 2.4 and 3.0 mls of gas per every 100 mg ingested, respectively. When 100 mg of the indigestible residues were actually fermented the total volumes of gas produced from the dry bean samples ranged from 33.0 ml for 'Nep-2' to 24.0 ml for 'Seafarer'. The rates of fermentation ranged from 0.74 ml of gas per hour for 'Proseed' to 0.41 ml of gas per hour for 'Seafarer'. In the cereals, wheat and barley produced 27.0 ml of gas whereas oats produced 26.2 ml of gas. The rates of fermentation ranged from 1.56 ml of gas per hour for oats to 1.37 ml of gas per hour for wheat and pearled barley.

Table 21. Proximate composition of the indigestible residue from dry bean and cereal flour (nonhomogenized).[a]

Sample	Protein[c]	Starch[d]	Fiber[e]	Ash[f]
Beans				
Sanilac	12.9 ±0.07	18.0 ±0.64	59.9 ±1.23	9.2 ±0.3
Seafarer	13.2 ±0.34	21.9 ±0.78	56.7 ±0.95	8.2 ±0.4
UI-114	22.2 ±0.14	23.2 ±1.27	47.4 ±1.02	7.2 ±0.4
Nep-2	25.6 ±2.02	20.0 ±0.42	45.2 ±0.65	9.2 ±0.4
San Fern	24.6 ±0.18	16.4 ±0.49	52.8 ±0.45	6.2 ±0.3
Fleetwood	18.7 ±0.39	23.8 ±4.24	50.2 ±0.84	7.3 ±0.8
Proseed	19.3 ±0.18	20.6 ±1.13	53.9 ±1.23	6.2 ±0.2
Cereals				
Wheat	7.9 ±0.04	17.0 ±0.20	64.1 ±1.10	11.0 ±0.2
Barley	9.8 ±1.32	23.0 ±0.85	57.1 ±0.36	10.1 ±0.1
Oats	11.4 ±0.45	18.0 ±1.13	54.6 ±1.02	7.2 ±0.4

[a] Mean ±S.D.
[b] Dry basis
[f] $n=2$, [c],[e] $n=3$, [d] $n=4$

SUMMARY

The major reasons for the avoidance of grain legumes is its potential to create abdominal discomfort and the elimination of intestinal gas (flatulence). These problems stem from the fermentation of the indigestible residue by colonic bacteria with gas as a by product. Studies were designed to determine what components of dry beans are indigestible and and could therefore be potential flatus factors.

Parents and progeny of an 8 X 8 diallel cross were evaluated to ascertain if genetic variability for low molecular weight sugars in beans, namely sucrose, raffinose and stachyose existed. The various sugars were extracted using 80% ethanol:water and anaylzed using HPLC. The extracts were injected onto an Alltech 600 CHC carbohydrate column using an elution solvent of acetonitrile:water 70:30 (v/v) and a refractive index detector. The samples were quantitated using external standards and repetitive injection techniques. Data were analyzed by 2 way analysis of variance for a randomized complete block design. Results showed significant and highly significant differences among the parents for raffinose and stachyose, respectively. Differences in sucrose content were nonsignificant as were the sampling and determination mean squares. In the 56 crosses sucrose, raffinose and stachyose contained were significantly different at the 1% level. The sampling and determination mean squares were not significantly different.

Samples of dry beans (Phaseolus vulgaris) and controls of cereals were digested in-vitro for the removal of digestible protein and

starch. The remaining residue (indigestible residue) was analyzed for indigestible protein and starch, for soluble sugars and for the various fiber components. Protein residue remaining after digestion varied from 16.1% for 'Seafarer' to 29.5% for 'San Fernando'. After digestion the protein in the cereal samples varied from 5.8% for pearled barley to 14.5% for corn. Indigestible starch content of the dry bean samples were from 13.7% for 'Seafarer' to 21.4% for 'Fleetwood'; while in the cereals, values ranged from 10.8% for corn to 19.0% for whole wheat. The dry bean samples contained between 2.18 to 2.94% stachyose and 0.34 to 0.53% raffinose. Raffinose and stachyose were not observed in the cereals. Dietary fiber content for the dry bean samples ranged from 17.8% for 'UI-114' to 19.9% for 'Sanilac' and 'Proseed'. In the cereal samples, pearled barley had the highest dietary fiber at 14.8% and corn the lowest at 8.6%.

Dry beans and cereals were analyzed for components which could escape digestion and absorption in the small intestine. The quantity of indigestible materials which would be expected to reach the colon and which would be expected to be fermented were multiplied by the gas volume per unit of indigestible component. The percentages of the original sample which were fermentable ranged from 24.7 mg/100 mg for 'Nep-2' to 27.3 mg/100 mg for 'Proseed'. The indigestible residue from Nep-2 could produce 5.3 ml of gas and the indigestible residue from Proseed. The indigestible residue from cereals ranged from 9.9 mg/100 mg for oats 6.1 mg/100 mg for pearled barley. The indigestible residue from oats could produce 2.4 ml of gas and the indigestible residue from pearled barley could produce 4.0 , which could produce 4.0 ml of gas.

When 100 mg of the ingestible residues were actually fermented the total volumes of gas produced from the dry a bean samples ranged from 33.0 ml for 'Nep-2' to 24.0 ml for 'Seafarer'. The rates of fermentation ranged from 0.74 ml per hour for 'Proseed' to 0.41 ml per hour for 'Seafarer'. In the cereals wheat and barley produced 27.0 ml of gas where as oats produced 26.2 ml of gas. The rates of fermentation ranged from 1.56 ml of gas per hour for oats to 1.37 ml of gas per hour for wheat and pearled barley.

RECOMMENDATIONS FOR FURTHER RESEARCH

There are a number of further studies that could be pursued that relate to the indigestibility and fermentability of dry beans:

1) Recross the progeny of the breeding experiment that showed low and high quantities of raffinose and stachyose. This would continue to establish if the quantity of soluble sugars can be manipulated by breeding techniques.

2) Correlate the in-vitro digestion results to an in-vivo digestion by possibly using ileo-cecal cannulated pigs or humans.

3) Establish the role that lectins, tannins and phytic acid play in the digestibility of starch and protein and the morphological changes in the gastrointestinal tract that may occur due to the presence of these components.

4) Determine if components in dry beans (such as lectins, tannins and phytic acid) play a factor in transit time of food through the GI tract.

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APPENDIX

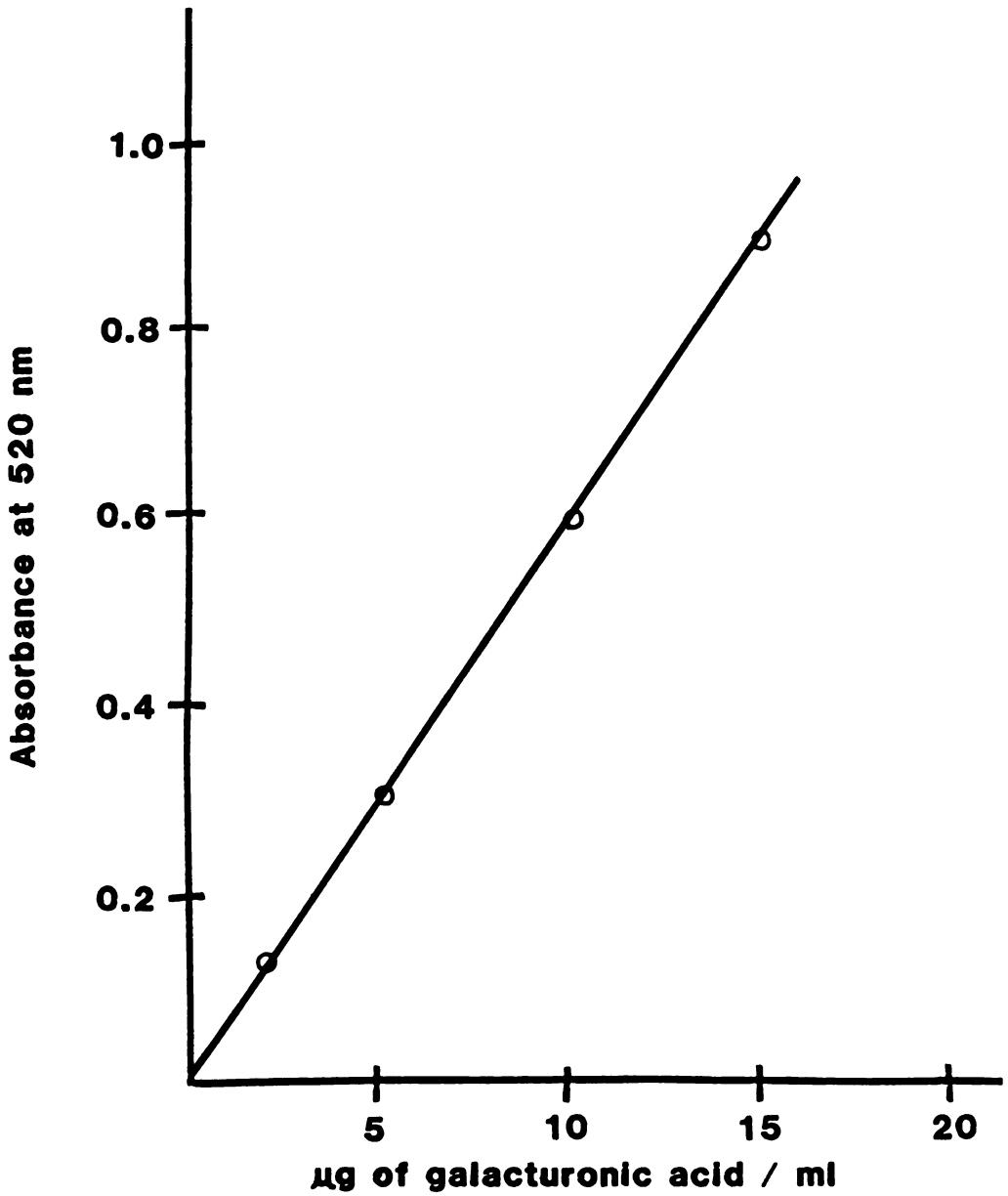

Figure 15. Relationship between galacturonic acid concentration and absorbance at 520 nm using m-phenylphenol.

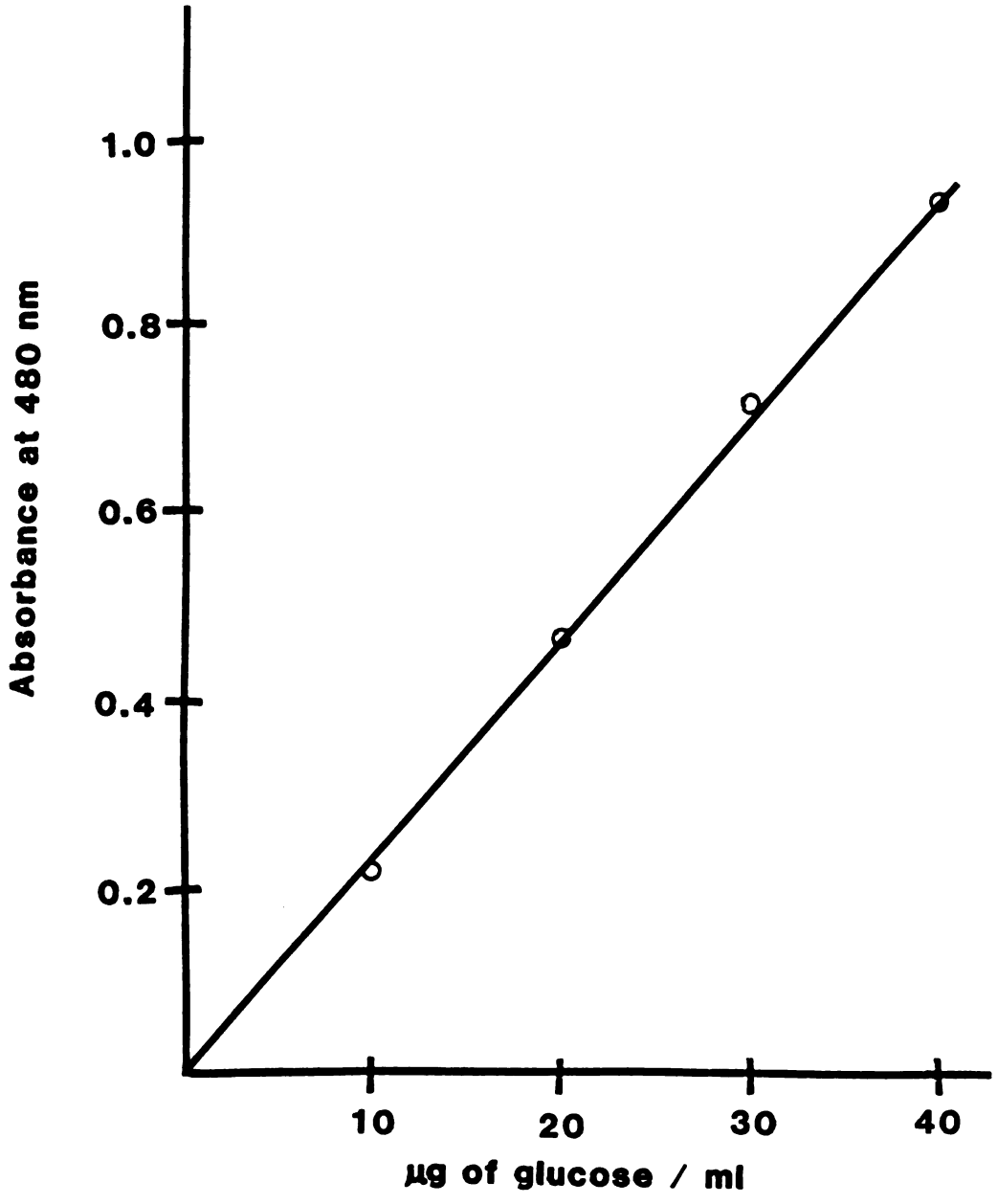

Figure 16. Relationship between glucose concentration and absorbance at 480 nm using phenol.