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Composition and Flatulence-producing Potential of Commonly Eaten Nigerian and American Legumes

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ABSTRACT

Sixteen samples of dry, mature legume seeds representing eight species purchased in markets either in Port Harcourt, Nigeria, or Griffin, GA, were cooked in boiling water until tender and freeze-dried along with cookwater. Flatulence potential measured as ml H_2 produced per gram of legume solids consumed was measured with rats housed in glass life-support chambers designed to collect hydrogen while supplying oxygen and absorbing water and carbon dioxide. Hydrogen was quantified by GLC using a molecular sieve column. Mono-, di- and oligosaccharides were determined by HPLC. Starch content was measured as glucose in a YSI analyzer and digestibility was determined in vitro *with pancreatic amylase. Hydrogen production was positively correlated with contents of xylose and fructose as well as stachyose and an unknown thought to be verbascose, not correlated with raffinose, and negatively correlated with galactose and indigestible starch.*

INTRODUCTION

Although beans and peas are an excellent source of protein and water soluble vitamins, per capita consumption of beans and peas in the US and

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other industrialized countries has fallen considerably in recent years (Murphy *et al.*, 1972). Even in developing countries where pulses remain an important, relatively affordable source of dietary protein, their consumption is minimized by those who can afford to replace them with animal-derived foods (Hardin, 1979). This trend is related to the time and labor required to prepare the dry seeds and to the unpleasant physiological effects which may accompany their consumption. These factors combine to maintain low prices, and in concert, relegate pulses to a low status among consumers (Dovlo *et al.*, 1976; Aguilera & Stanley, 1985). These negative factors in turn exert a depressing effect on production of potentially valuable leguminous crops.

A major reason for avoiding legumes is the difficulty experienced in digesting them. Flatulence is the most common symptom associated with pulse consumption, but its social implications are overshadowed by more serious accompanying consequences (El Faki *et al.*, 1983; Onigbinde & Akinyele, 1983). Abdominal pain and diarrhea are often experienced by susceptible individuals, especially children, causing pulses to be avoided. Unfortunately those affected are often most at risk from malnutrition; and pulses may be the only affordable source of good quality protein available to them.

The production of flatus by monogastric animals is due to colonic fermentation of carbohydrates which escape breakdown in the stomach and small intestine. The oligosaccharides raffinose, stachyose, and verbascose which are common in legume seeds are thought to be the major producers of flatulence when those foods are consumed. These saccharides are comprised respectively of one, two and three galactose units joined together and to sucrose in α-D-1-6 linkages. Due to the lack of α-galactosidases in mammalian digestive systems, they pass into the colon where they may produce diarrhea, flatus gas and their attendant discomfort (Wagner *et al.*, 1976; Fleming, 1981). Fiber polysaccharides and indigestible starch have also been associated with flatulence (Geervani & Theophilus, 1981; Marthinsen & Fleming, 1982). Hydrogen is a major component of flatus and has been shown to be related to total flatus production (Wagner *et al.*, 1977).

The purpose of this research was to measure the flatulence-producing potential of a cross-section of leguminous seeds which are commonly consumed in West Africa and in the US, and to correlate it to seed composition.

MATERIALS AND METHODS

Sixteen samples of dry, mature legume seeds representing eight species commonly eaten in West Africa and the US (Table 1) were obtained from

TABLE 1
Legume Seeds Studied and Their Cooking Times

Legume genus, species	*Variety*	*Cooking time (min)*
Phaseolus vulgaris	Kidney bean	120
	Black turtle bean	120
	Pinto bean	135
	Navy bean	95
	Great northern bean	240
Phaseolus lunatis	Baby lima	70
	Green baby lima	140
	Large lima	120
Pisum sativum	Green split pea	45
Vigna unguiculata	African black bean(?)[a]	75
	Blackeyed pea	65
Cajanus cajan	Pigeon pea	120
Lens esculenta	Lentil	20
Sphenostylis stenocarpa	Yam bean 1	240
	Yam bean 2	240
Voandzeia subterranea	Bambara groundnut	660

[a] Genus and species not certain.

markets in Port Harcourt, Nigeria, or Griffin, Georgia, USA. Approximately 30 g of dry seed of each sample were cooked in 100 ml of boiling tap water until they were judged to be tender enough for eating (Table 1). Water levels were kept constant during cooking by the addition of boiling water when necessary. Cooked seed and remaining cookwater were frozen, freeze-dried, and milled on a Retsch Microjet mill (Retsch GmbH, Haan, West Germany, Model ZM1) equipped with a 0·5 mm screen.

Flatulence potential of legume seeds was determined in adult (250–400 g), female Sprague Dawley rats as volume of hydrogen (ml) produced per weight (g) of legume consumed. Five replicate determinations were made simultaneously for each sample. The apparatus and details of methodology are described in detail elsewhere (Phillips *et al.*, 1988). Hydrogen production was determined in individual life support systems (ILSS) which supplied oxygen and water *ad libitum* and absorbed CO_2 and water vapor. Animals were maintained in individual cages on a control diet (*ad lib.*) comprised of 20% casein, 66% corn starch, 8% soybean oil, and the required levels of vitamins and minerals when not participating in an experiment. Test diets consisted of cooked, freeze-dried, ground legume seed. Prior to an

experiment, five animals were fasted for 24 h then, along with approximately 8 g of diet, were introduced into ILSS chambers. After 18 h, duplicate samples of ILSS atmosphere were analyzed for hydrogen by gas chromatography (Hewlett Packard 5790A instrument equipped with a 4·88 m by 0·32 cm, 80–100 mesh 5A molecular sieve column operated at room temperature; thermal conductivity detector). Hydrogen production from control diet was determined by substituting it for legume sample.

Starch content of legume samples was determined according to the method of Budke (1984) using a YSI carbohydrate analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio 45387). Suspensions of legume samples containing ~2 g starch in 100 ml of water were autoclaved for 1 h at 121°C, then saccharified with a mixture of α-amylase and amyloglucosidase. The glucose resulting from this procedure was measured and sample starch content computed according to manufacturer's instructions. Starch digestibility was calculated from maltose released by porcine amylase following predigestion with pepsin as described by Kon *et al.* (1971). Maltose was quantified by the dinitrosalicylic acid method of Bernfeld (1955). Corn starch served as a standard.

Saccharides were extracted from an aqueous slurry of uncooked legume sample with a 1:1 mixture of chloroform:methanol (Havel *et al.*, 1977), concentrated under vacuum and made to volume. Extracts were analyzed on a Micromeritics high performance liquid chromatograph (Micromeritics, Inc., Norcross, GA) equipped with refractive index detector. Separation took place on a 220 × 4·6 mm aminopropyl column (amino-spheri-5, Brownlee Labs, Santa Clara, CA 95050) eluted with 70:30 (v/v) acetonitrile: water which contained 0·01% tetraethylenepentamine (Aitzemuller, 1978). Quantification was against authentic external standard sugars.

All computations were performed using SAS (1985) procedures. Selected hydrogen volume means were tested for differences by determining orthogonal contrasts between them. Relationships between flatus hydrogen produced and composition variables were determined by backwards stepwise regression to determine which variables contributed to the model at the 5% level of significance, followed by a GLM procedure to determine the final model.

RESULTS AND DISCUSSION

The flatulence potentials (ml H_2/g) of legume samples are presented in Table 2; the results of contrast analysis on hydrogen volumes in Table 3; carbohydrate content in Table 4; and regression coefficients for equations relating hydrogen production to composition in Table 5. Earlier work in this

TABLE 2
Hydrogen Production Following Ingestion of Cooked Legume

Legume genus, species	*Variety*	*Hydrogen produced (ml/g)*
Phaseolus vulgaris	Kidney bean	1·61
	Black turtle bean	1·52
	Pinto bean	1·38
	Navy bean	1·22
	Great northern bean	0·81
Phaseolus lunatis	Baby lima	3·04
	Green baby lima	2·26
	Large lima	2·00
Pisum sativum	Green split pea	1·60
Vigna unguiculata	African black bean	1·67
	Blackeyed pea	1·56
Cajanus cajan	Pigeon pea	1·91
Lens esculenta	Lentil	1·00
Sphenostylis stenocarpa	Yam bean 1	1·86
	Yam bean 2	1·75
Voandzeia subterranea	Bambara groundnut	0·29
	Control diet	0·15

laboratory and by others indicates that the degree of variation in flatus data is rather high (Fleming, 1981; Phillips *et al.*, 1988). Nevertheless, there were marked, significant differences among the legumes examined in this study. Cultivars of *Phaseolus lunatis* produced the greatest response followed by pigeon pea and yam bean. At the opposite end of the spectrum, bambara groundnut produced no more flatus hydrogen than the control diet. The other species were intermediate in hydrogen production, with the potential of common bean (*Phaseolus vulgaris*) cultivars varying from high to low. Calloway *et al.* (1971) found that lima beans (*P. lunatis*) and California small white (CSW) beans (*P. vulgaris*) produced comparable levels of flatus H_2 in contrast to earlier work which found the latter to be more flatulent. Differences in methodology make direct comparisons to the present work difficult; however, Wagner *et al.* (1976) found values of about 2·5 ml/g when CSW beans were evaluated in rats as 10–60% of the diet. These amounts are comparable to the present values for lima beans but higher than any observed for *P. vulgaris* cultivars. Fleming (1981), studying several of the same species studied in this experiment and using similar methods, observed similar trends and values in ~5 week old, Wistar male rats. In her work, 2 g of bean were combined with 1 g of control, rather than feeding legume alone,

TABLE 3
Results of Performing Orthogonal Contrasts Between Hydrogen Volume Means

Contrast	*Hydrogen produced level of significance*
All legumes vs control	**
Phaseolus lunatis vs control	**
Cajanus cajan vs control	**
Sphenostylis stenocarpa vs control	**
Vigna unguiculata vs control	**
Pisum sativum vs control	**
Phaseolus vulgaris vs control	**
Lens esculenta vs control	**
Voandzeia subterranea vs control	NS
Phaseolus lunatis vs *Phaseolus vulgaris*	**
Phaseolus lunatis vs *Vigna unguiculata*	**
Phaseolus vulgaris vs *Vigna unguiculata*	*
Kidney bean vs great northern bean	**
Kidney bean vs navy bean	NS
Great northern bean vs control	*

** Significant at $P < 0.01$.
* Significant at $P < 0.05$.
NS Not significant at $P < 0.05$.

and provided a total of 3 g to each animal. Kidney bean and navy bean were found to produce 1·76 and 1·65 ml/g, respectively (calculating from the data in Fleming, 1981), while lentil produced 0·74 ml/g; which was not different from control diet. *Pisum* cultivars, wrinkled and smooth field pea, produced 1·32 and 0·97 ml/g, respectively.

Saccharide contents of legumes examined in this study were comparable to, although somewhat lower than, those in the literature, especially for stachyose. In a review, Reddy *et al.* (1984) give stachyose contents of 2·4 to 4·0% for *P. vulgaris*, 2·0 to 3·6% for cowpeas, 1·9 to 2·7% for lentil. Values for raffinose were more similar to those found in the present study; 0·3–0·7% for *Phaseolus vulgaris* cultivars, 0·4–1·2% for cowpea, and 0·4 to 1·0% for lentil. Unfortunately, we were unable to locate a source of authentic verbascose, making it impossible to analyze these legume samples for that sugar. However, an unknown peak which eluted following stachyose is probably verbascose, and was identified/quantified as such. The stachyose standard curve was used to quantify the unknown as little variation in standard response between the lower homologs, raffinose and stachyose,

TABLE 4
Carbohydrate Composition of Legume Seeds[a]

Legume variety	*Monosaccharides*			*Disaccharides*		*Oligosaccharides*			*Starch*	
	Xylose (mg/g)	*Fructose (mg/g)*	*Galactose/ Glucose (mg/g)*	*Sucrose (mg/g)*	*Maltose (mg/g)*	*Raffinose (mg/g)*	*Stachyose (mg/g)*	*(Verbascose?)*[b] *(mg/g)*	*Content (%)*	*Digestibility (%)*
Kidney bean	ND	3·34	ND	10·15	ND	3·18	16·03	ND	33·5	58·3
Black turtle bean	3·28	4·59	ND	7·90	4·02	3·77	11·95	ND	34·3	43·9
Pinto bean	1·66	2·20	ND	8·61	2·64	2·48	10·17	ND	40·6	67·1
Navy bean	3·93	5·32	ND	13·69	5·03	4·52	11·90	ND	40·6	28·7
Great northern bean	3·20	4·22	5·48	17·71		4·91	13·67	ND	34·2	47·2
Baby lima	2·86	5·50	ND	10·12	3·74	4·88	20·65	ND	36·8	55·9
Green baby lima	ND	4·63	ND	9·83	ND	4·87	20·00	ND	38·2	47·2
Large lima	ND	3·96	ND	10·42	ND	3·90	12·39	ND	35·4	52·7
Green split pea	ND	1·11	ND	3·75	3·74	2·88	7·01	8·23	39·7	69·6
African black bean	4·86	ND	3·18	6·44	2·95	4·68	22·39	6·03	40·0	22·6
Blackeyed pea	1·67	0·72	ND	4·69	ND	2·88	16·54	3·76	39·0	58·8
Pigeon pea	11·56	5·29	7·10	15·09	10·82	ND	7·55	7·44	40·0	50·0
Lentil	ND	2·01	ND	7·76	3·12	2·81	13·64	6·05	42·2	40·5
Yam bean 1	ND	2·45	3·43	6·72	ND	4·60	24·11	2·56	40·7	54·9
Yam bean 2	ND	3·20	4·21	9·85	ND	6·18	23·47	ND	40·8	51·2
Bambara groundnut	ND	0·62	ND	55·64	2·35	4·37	ND	ND	27·3	46·3

[a] Means of 2–4 determinations.
[b] Unknown peak throughout to be verbascose.
ND, not detected.

TABLE 5
Relationship between Volume of Hydrogen Produced and Oligosaccharide-Indigestible Starch Content of Legumes
$VH_2 = f$(Xylose, Fructose, Galactose, Stachyose, Verbacose, Id Starch)

Variable	$R^2 = 0{\cdot}84$ *Parameter*	*Contribution to* R^2
Intercept	0·859	
Xylose	0·111	2
Fructose (mg/g)	0·152	17
Galactose (mg/g)	−0·157	2
Stachyose	0·086	37
(Verbascose?)	0·076	7
Id Starch	−0·061	19

were observed. The unknown compound was not detected in *P. vulgaris* samples, while it was found in substantial levels in *Pisum*, *Vigna* and *Lens* species. This pattern agrees with that observed for verbascose reported by Reddy *et al.* (1984), although the levels were generally lower. Sucrose levels found in the present study were also lower than those reported by Reddy *et al.* (1984), while starch contents were generally within the same range.

The possible relationships between hydrogen production and individual saccharides, total galactosides, total starch, indigestible starch, and cooking times were investigated using a backwards–stepwise regression procedure in which all possible variables are included in the original model and those making the lowest contribution to R^2 are removed one at a time until only those making a significant contribution at $P < 0{\cdot}05$ remain. The results of this procedure were surprising in some respects (Table 4). As expected, a positive correlation between hydrogen production and the contents of stachyose and the unknown suspected of being verbascose was found. The lack of correlation between raffinose content and flatulence is believed to be due to the relatively small variation in content of that sugar. Although monosaccharides are not usually associated with flatulence, xylose and fructose contents were positively related to hydrogen production in this study. Galactose and indigestible starch were negatively correlated with H_2 production. These results are similar to the findings of others in some respects and differ from them in others. Most other researchers have found a relationship between α-galactoside content of legumes and flatulence (Murphy *et al.*, 1972; Wagner *et al.*, 1976; El Faki *et al.*, 1983), but there is equally strong evidence that other factors are also important. Murphy *et al.* (1972) and Wagner *et al.* (1976) found the oligosaccharide-free residue of California small white beans to exert a powerful synergistic effect on

flatulence production by aqueous alcohol extracts of beans, implying a role for indigestible polysaccharides. Fleming (1981) found hydrogen production to be positively correlated to raffinose and stachyose, but negatively correlated to verbascose content. She also found positive correlations between hydrogen and glucan and pentosan contents, but a negative relationship with starch. It is suspected that the significant effect of xylose and fructose concentration found in the present work may be related to the levels of indigestible polysaccharides for which they serve as monomers.

Work in this laboratory (Nnanna & Phillips, 1988), as well as that of others (Murphy *et al.*, 1972; Wagner *et al.*, 1976), has shown a drastic reduction in flatulence potential by processes which reduce the levels of oligosaccharides. Thus, even if other seed constituents contribute to the problem, removal of oligosaccharides by processes which are economically and technologically feasible and which preserve functional and nutritional quality can reduce the problem and increase legume consumption.

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