Nutrient Composition of and Digestive Response to Whole and Extracted Dry Beans

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The distribution of nutrients between hot water diffusible and nondiffusible constituents of whole California small white (CSW), light red kidney, and baby lima dry beans was examined. For CSW, 15% of the solids were hot water diffusible and contained more than 90% of the sugars sucrose, raffinose, and stachyose, 12% of the nitrogen, 10% of the fat, 60% of the ash, none of the crude fiber, 60% of the thiamin, 80% of the niacin, and 0% of any detectable starch or precipitable protein of the whole beans. Protein efficiency ratios (PERs) for cooked extracted beans were the same or better than those for nonextracted beans. A 50% reduction in flatulence potential measured by hydrogen production was observed when rats were fed extracted beans. A significant reduction in subjective gastrointestinal distress was reported by human subjects interviewed 24 h after eating cooked extracted beans compared to cooked nonextracted beans.

Legumes are a major source of food in the world today with an annual production in excess of 100 million metric tons. They contain 20–50% protein that is generally low in sulfur amino acids and high in lysine. This amino acid profile complements that typical of the cereals (Tobin and Carpenter, 1978). A significant problem associated with use of this valuable food resource in developed countries is poor consumer acceptablility due to gastrointestinal discomfort and flatus production (Calloway et al., 1971; Cristofaro et al., 1974; Olson et al., 1981). The α-galactosides, principally raffinose and stachyose, have been identified as important contributors to these adverse physiological effects. The sugars are indigestible in the upper human digestive tract and are fermented in the large intestine by the intestinal microflora to produce significant amounts of hydrogen, carbon dioxide, and sometimes methane. Production of these gases results in flatulence and the problems associated with flatulence.

It is known that sucrose and the α-galactosides raffinose, stachyose, and verbascose in beans are fairly well solubilized in water. Cristofaro et al. (1974) suggested that a blanching–soaking procedure might reduce the content of these sugars in legumes. Ku et al. (1976) investigated the extraction of the α-galactosides during the cooking of whole soybeans. The objective of the work reported here was to find out how applicable a blanch–soak method would be for the removal of these sugars from common dry beans, how much of the beans nutrients might be lost in such a procedure, and whether such a removal of the α-galactosides would reduce flatulence both objectively (in rats) and subjectively (in humans).

EXPERIMENTAL SECTION

Materials. Two *Phaseolus vulgaris* cultivars, California small white (CSW) and light red kidney, and one *Phaseolus lunatus* cultivar, baby lima, were obtained locally. The beans were hand sorted to remove dirt and badly damaged beans before use. Reagent-grade chemicals were used in this study unless otherwise noted.

Preparation of Whole Beans for Subjective Evaluation. Dry beans generally contain from 8 to 17% moisture. For purposes of material balance, the terms dry beans or dry cooked material in this paper refer to the dry beans on a moisture-free solids basis. Tap water at room temperature was used unless otherwise noted. Beans were soaked and cooked in stainless steel vessels. For subjective evaluation, whole beans equivalent to 2100 g of dry solids were rinsed briefly under running water and then placed in water (3.0 mL/g of bean fresh weight). After the beans had soaked overnight (18–20 h), 18 g of table salt (Morton, not iodized) was added and the beans were boiled gently for 60–75 min. No soak or cook waters were discarded at any stage in the preparation of whole beans. The cooked beans were adjusted to a weight of 7500 g by either adding water or removing the pot lid to allow steam to escape. Aliquots of 250 g of the cooked beans were weighed into tared disposable plastic bowls for the subjective evaluation panel. This amount of cooked beans corresponded to 70 g dry weight of beans/serving.

Preparation of Extracted Beans for Subjective Evaluation. Whole beans were weighed out and rinsed as described under Preparation of Whole Beans for Subjective Evaluation and immediately added to boiling water (3 mL/g of bean fresh weight) and boiled 3 min. The container was covered, removed from the heat, and allowed to stand for 2 h. The soak water was poured off and water added. In this and subsequent soakings, all at room temperature, enough water was added to just cover the beans. After 2 h the second soak water was poured off. A third soaking of 2 h was followed by soaking overnight (14–16 h), followed by five rinses with water. The beans thus extracted were cooked by adding water to cover and 28.5 g of table salt and boiled gently for 75–90 min. The cooked beans were adjusted to a weight of 7050 g by the addition of water or allowing steam to escape. Aliquots of 235 g, corresponding to 70 g of original whole dry beans, were used for the subjective evaluation panel.

Preparation of Beans for PERs and Hydrogen Production in the Rat and Analytical Work. Whole and extracted beans were prepared according to the procedures described previously except salt was omitted. Combined total extracts were boiled for 30 min. The cooked preparations were frozen and lyophilized after which the dried cooked beans were blended in a Waring blender.

Proximate Analyses, Minerals, and Vitamins. Proximate analyses for total solids, Kjeldahl nitrogen, crude fat, crude fiber, and ash were determined by the standard AOAC (1975) methods. Minerals were determined by atomic absorption spectrometry (Perkin-Elmer Corp., 1976) or X-ray fluorescence spectrometry (Reuter, 1975). Thiamin was determined by a chemical procedure, and riboflavin and niacin were determined by microbiological procedures (Freed, 1966).

Analysis for Sucrose, Raffinose, and Stachyose. Analyses were done by high-pressure liquid chromatog-

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raphy (HPLC) using the following equipment obtained from Waters Associate, Milford, MA: U6K injector, M6000A pump, R401 differential refractive index detector, Model 730 data module, and a μBondapak Carbohydrate Analysis column preceded by a guard column packed with Corasil AX. Aqueous extracts (0.5–1.0 mL) of beans were filtered through 0.22-μm disposable filters (Millex, Millipore Corp.), and 10–25 μL of the clear filtrates were injected onto the column. Separation of the sugars was achieved by using a 76:24 (v/v) mixture of prefiltered acetonitrile (Burdick and Jackson Laboratories, Inc., Muskegon, MI) and water at 2 mL/min. Quantitation was accomplished by comparison with standard solutions containing known amounts of sucrose, raffinose, and stachyose.

Amino Acid Analysis. Amino acids were analyzed on a Durrum amino acid analyzer, Model D-500, following the procedures provided by the manufacturer based on the method of Spackman et al. (1958). For free amino acids, 6.6 mg of lyophilized bean extract was dissolved directly in 5.0 mL of buffer, the solution filtered, and 40 μL used for the analysis. For total amino acids 7.7 mg of lyophilized bean extract was hydrolyzed in 10 mL of 6 N HCl at 110 °C for 20 h. After removal of the excess HCl, the residue was dissolved in 5.0 mL of buffer, the solution filtered, and 40 μL used for the analysis.

Rat Feeding Experiments. PERs in rats were determined by standard AOAC (1975) methods for biological evaluation of protein quality. The diets contained 10% protein supplied entirely by the respective bean samples except as noted. Modified PERs were performed as described in the text.

Hydrogen produced by rats was determined by the procedure of Gumbmann and Williams (1971) as summarized by Wagner et al. (1976). Experimental diets consisted of up to 25% test substance mixed with basal diet. Preconditioned rats in metabolic chambers were fed 10 g of experimental diets. The following day the gas in the chambers was analyzed for hydrogen by gas chromatography. Control diets containing different amounts of ground whole cooked CSW beans were fed with each set of experimental samples. A linear dose–response curve (log milliliters of hydrogen vs. log percent CSW beans in the diet) was constructed and used to determine the percent of CSW in the diet equivalent to the hydrogen produced by the experimental sample in question. Comparisons to CSW on a weight basis (relative potency) were accomplished by dividing the percent CSW equivalent by the percent of the test sample actually fed in the diet. A diet containing no beans was also routinely fed in order to monitor the hydrogen resulting from just the basal diet.

Subjective Evaluation of Cooked Beans. Cooked whole beans and cooked extracted beans were fed to a panel of volunteers to obtain their responses concerning intestinal gas production and discomfort over the following 24-h period. Fifteen to twenty-five adult judges participated in each of three replications for each variety of beans. The judges were 24–64 years old, about equally divided on the basis of sex with normal American eating habits. Judges participated in the testing once each week, consuming the cooked beans at noon. No special selection was made for the panelists nor were any dietary regimes required with the exception that they were urged not to eat foods known to make them flatulent (i.e., beans) during the test period. Judges served as their own controls by receiving a questionaire the day before the testing. The questionaire asked for the number of flatus discharges during each 4-h period covered in the 24 h and an estimate of the overall discomfort experienced for the entire period on a scale of 1–7 with 4 as neither *more* nor *less* than normal. Another questionaire was issued at the time the beans were eaten to cover the following 24-h period.

RESULTS AND DISCUSSION

Preparation of Beans for Subjective Evaluation. Whole beans prepared as described became tender and generally acceptable after 60–75 min of cooking. To achieve the same qualitative tenderness for the extracted beans required 75–90 min of cooking. This is consistent with reports that the presence of salts decreases legume cooking time because of the involvement of the salts in the breakdown of the middle lamella (Sefa-Dedeh et al., 1978; Rizley and Sistrunk, 1979). Addition of small amounts of sodium chloride were made to make the beans more palatable to the panelists. In separate experiments where analyses were done on preparations to which salt had been added, no significant differences were noted in sugar and proximate analyses except in the ash figures which were increased, reflecting the presence of the added salt. Cooked extracted beans were slightly lighter in color and more bland in taste than cooked whole beans. Where cooked and extracted beans were subsequently incorporated into more conventional bean recipes, there was significantly less difference in appearance, texture, and taste.

Proximate Analyses. The proximate analyses for cooked whole and extracted beans are listed in Table I. The percent of the constituent present in the whole bean that remains after extraction is also listed. Proximate analysis and sugar, thiamin, and niacin analyses were also performed on the extracts (Table II). Recovery of constituents for all proximate analyses and sugars was 101 ± 2% and for the vitamins 97 ± 9%. Thus, the loss in total solids in extracted beans of 15, 18, and 17% for the three varieties is confirmed by the recovery of these amounts of the original bean solids in the dried extracts. The extraction procedure is specific for certain bean constituents and not for others. Fat and crude fiber values remain essentially unchanged while the water-soluble constituents containing nitrogen (12–14% of whole bean nitrogen) and inorganic salts as measured as ash (60–70% of whole bean ash) were removed.

Sucrose, Raffinose, and Stachyose. Over 90% of these sugars were removed from the beans in the extraction process (Table I). These sugars were recovered in the extract (Table II). The rate of release of the sugars into the soak water after boiling was time dependent, with about 75% released from the beans in the first 4 h at which time the temperature had returned to room temperature, 25 °C. Adding beans to larger volumes of boiling water (10 mL of water/g or more), boiling for 3 min, removing from the heat, and allowing to cool to room temperature overnight resulted in the same loss in sugars and total solids as the procedure described. In the use of sequential extracts, each extract was kept to a minimum (beans just covered with water), resulting in a smaller total extract volume. Other beans that gave similar results following these extraction procedures include garbanzo, soy, pinto, large lima, Jacobs Cattle, and blackeye.

The water extraction process removed 15–18% bean solids, of which sucrose and stachyose account for over 40%. Overnight soaking in water at 25 °C removed only about 2% total bean solids and insignificant amounts of sucrose or stachyose. Starch measured by I_2–KI or by glucoamylase digestion followed by testing for glucose was not found in the extracts. Prolonged initial boiling or too slow a rate of cooling did, however, solubilize starch, resulting in its appearance in the extract.

Table I. Analysis of Cooked Whole and Extracted Beans[a]

analysis	California small whites	light red kidneys	baby limas
proximate			
whole beans			
nitrogen, %	4.2	4.0	4.1
fat, %	2.1	1.5	1.1
fiber, %	6.0	4.3	3.9
ash, %	4.9	4.4	4.6
extracted beans			
total solids, %	100 (85)[b]	100 (82)[b]	100 (83)[b]
nitrogen, %	4.4 (88)	4.2 (87)	4.3 (86)
fat, %	2.2 (90)	1.8 (98)	1.3 (98)
fiber, %	7.3 (103)	4.9 (94)	4.7 (100)
ash, %	2.4 (42)	2.1 (39)	1.7 (31)
sugars			
whole beans			
sucrose, %	3.0	5.0	2.6
raffinose, %	0.6	<0.05	0.5
stachyose, %	3.9	3.5	4.9
extracted beans			
sucrose, %	0.05 (1)	0.85 (14)	0.06 (3)
raffinose, %	0.07 (10)	<0.02	0.03 (5)
stachyose, %	0.28 (6)	0.43 (10)	0.41 (7)
vitamins			
whole beans			
thiamin, mg/100 g	0.80	1.01	0.63
niacin, mg/100 g	1.2	2.9	1.46
riboflavin, mg/100 g	0.14	0.15	0.18
extracted beans			
thiamine, mg/100 g	0.26 (28)	0.57 (47)	0.19 (25)
niacin, mg/100 g	0.54 (38)	0.54 (15)	0.18 (10)
riboflavin, mg/100 g	0.043 (26)	0.08 (44)	0.05 (23)

[a] Dry weight basis. [b] Numbers in parentheses are the percentages of each component remaining based on the amount found in whole cooked beans.

Table II. Analysis of Cooked Bean Extracts[a]

analysis	California small whites	light red kidneys	baby limas
proximate			
total solids, %	98 (15)[b]	98 (18)[b]	98 (17)[b]
nitrogen, %	3.3 (12)	3.0 (14)	2.6 (11)
fat, %	1.5 (11)	0.9 (11)	0.2 (1.9)
fiber, %	0.3 (0.7)	1.5 (7)	0.6 (2.4)
ash, %	19 (58)	16 (61)	21 (76)
sugars			
sucrose, %	20 (99)	24 (86)	15 (98)
raffinose, %	3.6 (90)	<0.1	2.8 (95)
stachyose, %	24 (94)	18 (90)	27 (93)
vitamins			
thiamin, mg/100 g	3.4 (63)	2.7 (48)	2.6 (70)
niacin, mg/100 g	6.5 (81)	14 (87)	5.8 (68)

[a] Dry weight basis. [b] Numbers in parentheses are the percentages of the components extracted based on the amount found in whole cooked beans.

Vitamins and Minerals. Over 50% of the thiamin, niacin, and riboflavin in the whole cooked beans was lost during extraction (Table I).

The large loss of ash in the extracted beans (Table II) was reflected in reduced content of specific minerals (Table III). Less than 50% of the sodium, magnesium, potassium, and phosphorus was retained while over 50% of the calcium, iron, copper, zinc, and manganese was retained in the extracted beans. Over 70% of the large amount of potassium found in these beans was removed by the extraction. In separate experiments (data not shown), analysis of the extracts for these minerals accounted for the losses from the extracted beans.

Amino Acid Analysis. Any nitrogen lost in the extract could represent a significant nutritional loss in terms of protein. Experimentally none of the extracted nitrogen was recoverable as precipitable protein by conventional procedures such as the use of ethanol or trichloroacetic acid. A closer examination of the extract from CSW beans showed that it contained free amino acids, principally aspartic acid, glutamic acid, and arginine, amounting to 3.3% of the dry weight of the extract (Table IV). After acid hydrolysis the recovered amino acids amounted to 9.4% of the extract and 1.4% of the dry weight of the original dry beans. The total recovered amino acids now accounted for 50% of the nitrogen in the extract. The hydrolysis resulted in increases in aspartic acid, glutamic acid, arginine and ammonia. The large increase in the glutamic acid and ammonia suggests the presence of glutamine or glutamine peptides in the original extract. No methionine was detected in either the extract or the hydrolyzed extract. The relative quantities of amino acids present in the extract do not resemble those for CSW bean protein, and investigation of them and their nutritional role may be warranted, particularly in view of the PER data discussed in the following section.

PER Studies. The PER values (Table V) for extracted beans (diets 2, 7, and 12) were all equivalent to or greater than those for whole beans (diets 1, 6, and 11). In the case of CSW, the difference was statistically significant ($P < 0.01$). The protein content for these diets (10%) was based on Kjeldahl nitrogen × 6.25. The results show that the nitrogen in the extracted CSW beans represented a more valuable protein source (in terms of PER) than that of whole beans and implies that the nitrogen discarded was of lesser value. In the other two bean varieties, the differences in PER between whole and extracted beans were not statistically significant.

Two other diets were fed in attempts to assess the nutritional value of the nitrogen lost during extraction. In the first (diets 3, 8, and 13) the extractable nitrogen that was discarded was considered nonprotein nitrogen (NPN) and was subtracted from the nitrogen value for whole

Table III. Mineral Contents of Cooked Whole and Extracted Beans[a]

analysis	California small whites	light red kidneys	baby limas
whole beans			
sodium, %	0.05	0.10	0.08
magnesium, %	0.19	0.15	0.21
calcium, %	0.18	0.12	0.12
potassium, %	1.8	1.6	1.8
phosphorus, %	0.50	0.55	0.41
iron, ppm	82	82	80
copper, ppm	8	10	6
zinc, ppm	25	37	34
manganese, ppm	15	12	23
extracted beans			
sodium, %	0.02 (34)[b]	0.05 (39)[b]	0.03 (31)[b]
magnesium, %	0.08 (35)	0.08 (46)	0.06 (24)
calcium, %	0.18 (85)	0.11 (77)	0.09 (59)
potassium, %	0.61 (29)	0.59 (30)	0.48 (22)
phosphorus, %	0.26 (45)	0.29 (43)	0.25 (51)
iron, ppm	69 (71)	57 (56)	52 (54)
copper, ppm	6 (64)	7 (56)	5 (69)
zinc, ppm	16 (53)	28 (63)	27 (65)
manganese, ppm	15 (84)	13 (90)	19 (69)

[a] Dry weight basis. [b] Numbers in parentheses are the percentages of each component remaining after extraction of whole beans.

Table IV. Amino Acid Content of CSW Extract

amino acids	free amino acids in extract,[a] %	total after acid hydrolysis,[a] %
Asp	0.52	1.64
Thr	0.33	0.38
Ser	0.48	0.28
Glu	0.63	3.6
Gly		0.29
Ala		0.23
Ile		0.12
Leu		0.39
Phe		0.14
His	0.09	0.28
Lys		0.49
NH_3		0.51
Arg	1.29	1.6

[a] Percent in extract on dry weight basis.

beans. By use of this adjusted nitrogen figure, a new diet for whole beans was prepared that now contained more whole bean meal than the original. If the NPN does not contribute to PER, then this adjusted whole bean diet should give PER values equivalent to those for extracted beans (diets 2, 7, and 12). Experimentally this was true for whole CSW and for light red kidney. For baby lima there was a significant increase in PER, suggesting the possible loss of nitrogen having some protein value.

In the second special diet (diets 4, 9, and 14), the amount of extracted meal was equivalent to the yield from whole beans fed in diets 1, 6, and 11. The question addressed in this case was what is the effect of the loss of 11–14% of the nitrogen from a given amount of bean meal. In all three cases, no significant differences were found in PER between whole or the equivalent amount of extracted beans. Thus, with the possible exception of baby lima beans, no evidence was obtained that indicated that the amino acids and peptides lost during extraction contributed to PERs.

Rat Assay for Hydrogen Production. Hydrogen production from rats fed extracted beans was less than 50% of that from rats fed whole beans (Table VI). The extracts from the beans, fed in amounts equivalent to what was extracted from whole beans, produced about 50% of the hydrogen from the whole beans for CSW and baby limas but less than 25% of that from whole light red kidneys. When cooked extracted beans and cooked extract were recombined in their original proportions, the amounts of hydrogen produced were not significantly different from that produced by the whole beans. When the amount of stachyose and raffinose found in whole CSW beans or bean extract was fed separately (diet 6), it caused rats to produce the same amount of hydrogen as the bean extract (diet 3). Adding this same amount of stachyose and raffinose to extracted beans (diet 5) produced the same amount of hydrogen from the rats as whole beans (diet 1) or reconstituted beans (diet 4).

The Gumbmann rat assay has been an excellent tool with which to study the effects of beans and bean fractions on the production of hydrogen by rats as a presumptive test for flatulence. In some instances the data have suggested the possibility of synergistic responses between different bean fractions (Olson et al., 1975; Wagner et al., 1976). In these experiments the results for light red kidney fractions are significantly lower than those from whole or reconstituted beans. The effect is also seen in comparing percent CSW equivalents for the whole beans with the sum of the equivalents for the light red kidney fractions.

Relative potency of the diets to CSW again shows that extracted beans are less potent in producing hydrogen than whole beans. The very high potency of the stachyose and raffinose (diet 6) identifies these sugars as significant contributors to the problem. These sugars make up 27% of the weight of the extract. Recalculating the potency of diet 3 on the basis of the amount of stachyose and raffinose contained in the extract ($0.27 \times 3.75 = 1.0$) gives a relative potency for this diet of 9.9, experimentally the same as the 9.6 for diet 6 which contained only stachyose and raffinose.

Using bean diets, Wagner et al. (1977) reported a positive correlation between measurement of hydrogen production in the rat and flatulence in man. The flatulence potential of dry beans then appears about equally divided between the extractable and nonextractable components. While the compounds causing flatulence production in the extracts are the α-galactosides, the compounds causing flatulence in the extracted beans are not as clearly identified. Protein components, whether from dry beans (Olson et al., 1975) or soy (van Stratum and Rudrum, 1979), are not significant contributors. As suggested by several groups (Hellendorn, 1976, 1979; Olson et al., 1975, 1981; Fleming, 1981), hemicellulose found in the water-insoluble portion of beans are reasonable nonabsorbable

Table V. Effect of Extraction on PERs of Beans[a]

dietary source[b]	PER actual[c]	PER adjusted
California small white beans		
(1) whole	1.58 ± 0.03 C	1.27
(2) extracted	2.11 ± 0.06 B	1.70
(3) whole (modified, total *N* minus extractable *N*)	2.02 ± 0.08 B	1.62
(4) extracted (modified, equivalent to yield from diet 1)	1.70 ± 0.04 C	1.37
(5) ANRC casein control	3.11 ± 0.08 A	2.50
light red kidney beans		
(6) whole	1.33 ± 0.05 BC	1.03
(7) extracted	1.58 ± 0.06 B	1.22
(8) whole (modified, total *N* minus extractable *N*)	1.58 ± 0.06 B	1.22
(9) extracted (modified, equivalent to yield from diet 6)	1.20 ± 0.12 C	0.93
(10) ANRC casein control	3.23 ± 0.10 A	2.50
baby lima beans		
(11) whole	1.91 ± 0.07 C	1.41
(12) extracted	2.04 ± 0.04 C	1.50
(13) whole (modified, total *N* minus extractable *N*)	2.41 ± 0.09 B	1.78
(14) extracted (modified, equivalent to yield from diet 11)	1.79 ± 0.06 D	1.32
(15) ANRC casein control	3.39 ± 0.10 A	2.50

[a] Used five Sprague-Dawley, male, 21-day-old rats for each diet. Results reported for three separate PER analyses: compare sets 1-5, 6-10, and 11-15 internally; all analyses were 28 days. Adjusted values to ANRC casein control equal 2.50. [b] All diets except 4, 9, and 14 contained 10% protein. Diets 1, 2, 5-7, 10-12, and 15 used $N \times 6.25$ for protein. Diets 3, 8, and 13 used (total N minus extractable N) $\times$ 6.25 for protein. Diets 4, 9, and 14 used amount of beans left after extraction of the amount of beans used in diets 1, 6, and 11; these diets contained 8.8, 8.7, and 8.6% protein, respectively. [c] Mean ± SE from Duncan's (1955) multiple range test. Means without a capital letter in common are significantly different; $P < 0.01$. Five rats per group.

Table VI. Effect of Different Bean Preparations on the Production of Hydrogen by Rats[a]

prepn added to diet	% in diet	mL of H_2 ± SE	significant difference[b]	% CSW equiv in diet[c]	rel potency to CSW[d]
California small white					
(1) whole beans	25.00	9.0 ± 0.8	A	23.7	0.95
(2) extracted beans	21.25	4.3 ± 0.6	B	10.3	0.48
(3) extract from diet 2	3.75	4.1 ± 0.5	B	9.9	2.64
(4) recombined diets 2 + 3	25.00	8.8 ± 1.0	A	21.9	0.88
(5) diet 2 + stachyose	22.27	9.5 ± 0.8	A	24.8	1.11
(6) stachyose + raffinose	1.02	4.5 ± 0.7	B	9.8	9.61
light red kidney					
(7) whole beans	25.00	8.3 ± 0.7	A	28.3	1.13
(8) extracted beans	20.39	3.5 ± 0.5	B	10.3	0.57
(9) extract from diet 8	4.71	2.1 ± 0.4	C	5.2	1.25
(10) recombined diets 8 + 9	25.1	7.4 ± 1.3	A	19.4	0.77
baby lima					
(11) whole beans	25.00	9.4 ± 1.1	A	26.3	1.05
(12) extracted beans	21.27	3.3 ± 0.4	B	5.7	0.27
(13) extract from diet 12	3.73	5.5 ± 0.6	B	12.5	3.35
(14) recombined diets 12 + 13	25.00	10.0 ± 1.6	A	25.8	1.03

[a] Used Sprague-Dawley, male, 31-day-old rats. Results reported from three separate runs: compare sets 1-6, 7-10, and 11-14 internally. Basal diet responses: for set 1-6, 1.0 ± 0.2 mL, for set 7-10, 0.8 ± 0.1 mL, and for set 11-14, 1.6 ± 0.2 mL. [b] Duncan's (1955) multiple range test: means without a letter in common are significantly different; $P < 0.05$; N = 10-13. Statistical analysis performed on log transformed data. [c] Calculation based on a least-squares dose-response curve; see the text. [d] Calculated as (percent CSW equivalent)/(percent test sample in diet).

carbohydrates likely to be fermentable in the colon and contributors to this problem.

Subjective Evalution of Cooked Whole and Extracted Beans. Objective determination of flatus composition and volume and breath hydrogen measurements are valuable methods with which to identify those food components that produce such responses. There are, however, many problems connected with these measurements from people, and the measurements themselves do not answer the question as to whether one preparation is preferred over another on the basis of producing less flatus and/or gastrointestinal discomfort. In this study panelists reported fewer average discharges and less overall discomfort after eating extracted beans in each of the three replications for the three bean varieties tested. Averages for all judges for each replication, the means for the three replications for each variety and the mean for all varieties are shown in Table VII. Overall reduction in the number of gas discharges was 27%. Not surprisingly, individual variations in the number of discharges varied widely. However, some of the panelists who participated in all nine replications gave consistently lower responses for all of the extracted samples while no panelist gave consistently lower responses for the control samples. Overall discomfort responses were also lower in every case for the extracted beans compared to whole beans.

The nonparametric sign test was applied to the data, and the results are shown in Table VIII. From this analysis, it is clear that both number of discharges and overall discomfort were significantly less for extracted beans compared to whole beans ($P < 0.05$ or better) for all three varieties. Thus, the extraction process significantly reduces the number of reported gas discharges and the discomfort caused by eating cooked beans.

There are many factors influencing gas production from fermentation of food components in the colon. For ex-

Table VII. Average Number of Gas Discharges and Overall Discomfort during 24 Hours Immediately after Each Feeding of Cooked Beans (70 Grams Dry Basis)

		no. of discharges				av overall discomfort[a]	
		whole beans		extracted beans			
variety	replication	av	range	av	range	whole beans	extracted beans
California small white	1	15.0	0-37	13.8	1-37	5.5	5.3
	2	20.2	1-44	16.7	0-32	5.6	5.0
	3	17.6	5-42	15.5	1-29	5.4	4.8
mean (3)[b]		17.6		15.3		5.5	5.0
light red kidney	1	19.5	0-61	12.2	1-30	5.6	5.0
	2	16.6	2-54	11.4	0-31	5.3	4.3
	3	14.6	1-52	13.1	4-32	5.1	4.7
mean (3)		16.9		12.2		5.3	4.7
baby limas	1	16.7	0-45	11.0	0-32	5.4	4.5
	2	18.6	0-68	11.2	0-29	5.5	4.8
	3	19.6	4-48	10.7	3-31	5.9	4.2
mean (3)		18.3		10.9		5.6	4.5
mean (all)[c]		17.6		12.8		5.5	4.7

[a] Rated on a scale of 1-7 with 1 = much less than normal, 7 = much more than normal, and 4 = neither more nor less than normal. [b] Mean for three replications. [c] Mean for all varieties and replications.

Table VIII. Sign Test Analysis[a] of Individual Results for Gas Discharges and Overall Discomfort from Three Varieties of Dry Beans

	no. of cases in which no. of gas discharges were greatest for		no. of cases in which greater discomfort was indicated for	
variety	whole beans	extracted beans	whole beans	extracted beans
California small white	30	14	28	7
light red kidney	36	16	34	13
baby limas	43	10	42	7

[a] Beyer (1968). Differences were significant at the 5% level for all sets of observations.

ample, results reported in this paper refer to a sudden influx of malabsorbed carbohydrate into the colon. Continued addition of such material into the colon results in changes in fermentation patterns including an increase in low molecular weight fatty acid production and decreases in pH and hydrogen production (Perman et al., 1981). People on diets which regularly include significantly high levels of malabsorbed carbohydrates, then, may respond differently from people consuming such a diet only infrequently. Such evidence has been reported for hydrogen production in the rat by Gumbmann and Williams (1971). There may also be threshold levels of malabsorbed carbohydrate below which intestinal microflora respond in a different manner compared to how they respond to higher levels and in so doing effect both objective and subjective responses.

CONCLUSION

The data presented in these studies demonstrate the feasibility of removing the indigestible α-galactosides from whole dry beans by using a blanch–soak treatment followed by discarding the soak water. Significant protein losses are small or nonexistent, and the procedure should be applicable to both home use and food industry application. The procedure significantly reduced flatus production as measured objectively by hydrogen production in the rat. Subjectively, a test panel reported less discomfort from beans prepared in this way compared to beans prepared without the blanch–soak treatment.

ACKNOWLEDGMENT

We gratefully acknowledge the contribution of Dr. D. Guadagni for the subjective evaluation of the samples and A. Noma, G. McDonald, and R. Young for some of the analyses.

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Received for review June 8, 1981. Accepted October 13, 1981. Reference to a company and/or product named by the U.S. Department of Agriculture is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable. Presented, in part, at the Second Chemical Congress of the North American Continent, 180th National Meeting of the Americal Chemical Society, Las Vegas, NV, Aug 1980, Division of Agricultural and Food Chemistry.

Characterization of Storage Proteins Extracted from *Avena sativa* Seed Protein Bodies

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Protein bodies have been isolated from *Avena sativa nuda* (cv. Rhea) caryopses by differential centrifugation. Their protein content has been compared to proteins extracted from the meal. Albumins, globulins, prolamins (avenins), and glutelins have been sequentially extracted and characterized both quantitatively and qualitatively by electrophoresis and amino acid analysis. Avenins have also been compared by ion-exchange chromatography. The isolated protein bodies were free from starch, and the proportion of their proteins was different from that of proteins extracted from meal. The protein body proteins are mainly composed of acetic acid soluble glutelins and globulins. Extracted from protein bodies, the albumins, globulins, and acetic acid soluble glutelins form a continuous family contrary to the meal-extracted corresponding groups. The avenin polypeptide chains are all located within protein bodies.

Oat seed storage proteins (Kim et al., 1978), as compared to other cereals, exhibit some peculiarities. The proportion of avenins (the oat prolamins) is quite low compared to prolamins from other species such as wheat gliadins, barley hordeins, or maize zeins (Mossé, 1966). Avenins are not as characteristic as other prolamins, when compared to other storage protein groups on the amino acid composition and on electrophoretic bases. As for other prolamins, avenins belong to the alcohol-soluble protein group, but all polypeptide chains extracted by ethanol mixtures are not typical prolamins. Their definition needs further characterization by electrophoresis and amino acid analysis (Kim et al., 1978). Consequently, we call avenin (or prolamin) the typical fraction of the alcohol-soluble group and not all the proteins extracted by ethanol mixtures.

The interest of prolamins does not only consist in the fact that they represent one of the major storage protein groups which conditions the nutritional value of the seed but also avenins have been shown to constitute a useful tool for phylogenetic studies of the genus *Avena* (Kim et al., 1979b; Kim and Mossé, 1979).

Storage proteins and especially prolamins are located within typical storage organelles, called protein bodies, which occur in the seed endosperm (Pernollet, 1978). Nevertheless, some polypeptide chains belonging to the albumin and globulin groups may be deposited outside these organelles. Within cereal endosperm, one can usually distinguish two different kinds of protein bodies. In the aleurone layer, the protein bodies, also called aleurone grains, exhibit globoid inclusions containing phytin. In the starchy endosperm no such inclusions (nor as much phytin) can be detected within the protein bodies.

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Few studies have dealt with the characterization of oat protein bodies. Sraon (1972) has evidenced protein bodies within oat starchy endosperm, whereas Pomeranz (1972) has failed in locating protein bodies in this tissue. Buttrose (1978) has used electron dispersive X-ray analysis to characterize the protein bodies in the seed embryo of *Avena sativa*. This technique has widely been developed for seed studies. It is a proper tool to differentiate protein bodies within the starchy endosperm from those located in the aleurone layer of *A. sativa* seed (Pernollet and Mossé, 1980). Moreover, Pernollet and Mossé have shown that protein bodies are still present at maturity in the starchy endosperm of oat (Aveneae), contrary to the case in cereals belonging to the *Triticeae* tribe like wheat, barley, or rye.

The isolation of protein bodies from oat seeds has never been performed, whereas this investigation has already been done in other cereals (Pernollet, 1978). This paper describes the isolation of protein bodies from *A. sativa* caryopses, the cytological location of storage protein groups, and the relationships between these groups.

Differential centrifugation has deliberately been used to isolate protein bodies because it is possible to obtain large amounts of materials, which compensates for the low yield of the homogenization steps. We have preferred a gentle homogenization procedure to prevent protein bodies from alteration.

MATERIALS AND METHODS

Plant Material. The seeds of naked oat (*Avena sativa nuda*, cv. Rhea) were obtained from the INRA Plant Breeding Station of Rennes. Mature dry seeds were used throughout this investigation. The nitrogen content of the meal, obtained by the use of a cutting mill, was 2.39% on a dry weight basis.

Optical Microscopy. A M 20 Wild optical microscope equipped with phase contrast and polarized light measurements was used to follow protein body isolation.

0021-8561/82/1430-0032$01.25/0 © 1982 American Chemical Society