Effect of Processing on Composition and *Tetrahymena* Relative Nutritive Value of Green and Yellow Peas, Lentils, and White Pea Beans[1]

K. R. DAVIS[2]

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ABSTRACT

Moisture, fat, ash, tannin index, total phosphorus, *Tetrahymena* relative nutritive value (T-RNV), and in vitro digestibility of legumes were significantly influenced by type of legume. Moisture, fat, ash, protein, phosphorus, phytic acid, catechin equivalents, digestibility, and T-RNV were significantly influenced by treatment. Protein content had a significant ($P = 0.0001$) simple negative correlation with moisture and simple positive correlation with fat, ash, phytic acid, phosphorus, and catechin equivalents. Multiple stepwise maximum R^2 analysis, however, showed a significant negative association with phytic acid, tannin index, and catechin equivalents and positive correlation with phosphorus. Those four variables accounted for 93.06% of the variability in protein content. Digestibility was negatively correlated with ash and positively correlated with moisture and protein by stepwise maximum R^2 analysis. The model accounted for only 40% of the variability in the digestibility. T-RNV had a significant negative simple correlation with digestibility. A highly significant negative multiple correlation with protein and phosphorus and a positive correlation with ash was found. Yellow peas had the lowest average T-RNV (33%) and Aurora beans the highest (69%) versus casein (100%). Precooked flours had the lowest T-RNV (35%) and protein concentrates the highest (51%). A significant ($P = 0.0001$) simple negative correlation between T-RNV and digestibility was found ($r = -0.5122$). The digestibility and T-RNV were sensitive enough to detect changes in protein quality due to type of legume and treatment. Ash, protein, moisture, and phosphorus all had statistically significant effects on protein quality and may have to be considered when establishing a model to relate with the rat protein efficiency ratio.

Legumes, noted for their protein content, are frequently used as a dietary protein source. Protein nutritional quality is dependent on the quantity and availability of the essential amino acids, and legumes are generally limiting in the sulfur-containing amino acids. The amino acid composition and, presumably, the protein quality in beans, is altered by varietal differences (Chang and McAnelly 1961, Davis et al 1979, Sgarbieri et al 1979) and by growing location (Chang and McAnelly 1961).

Presently, the small differences in protein quality that result from breeding programs, agronomic practices, or kinds of processing are not easily measured. McCurdy et al (1978) suggested that an assay using the protozoan, *Tetrahymena pyriformis* W might be suitable for measuring these small differences.

Legumes contain such antinutritional or toxic factors as protease inhibitors, phytic acid, tannins, and lectins, all of which have the potential to affect protein availability or to be toxic. For instance, results of a *Tetrahymena* assay on lentil protein were lower than expected from the amino acid analyses (McCurdy et al 1978). Perhaps antinutritional or toxic factors in lentils made the protein unavailable or inhibited the growth of the organism.

Barré (1956) demonstrated some inhibition of proteolytic activity by phytic acid. Dryden (1977), however, found no effect on in vitro digestibility and no effect on *Tetrahymena* growth when phytic acid was added to casein. Phytic acid is a powerful chelating agent for divalent cations and has the potential to interfere with mineral availability. Calcium is an essential cofactor for the proteolytic enzymes. Phytic acid could affect digestibility by chelating calcium or by binding with the substrate or enzyme proteins. A 1% level of phytic acid in the diet has been reported to interfere with mineral metabolism in rats (Erdman and Forbes 1977). Pinto beans have 0.96% phytic acid (Makower 1970) and navy beans 1.78% (Harland and Prosky 1979). Vose et al (1976) reported that oil, phytic acid, soluble sugars, phosphorus, zinc, and calcium were partitioned into the protein concentrate fraction of pin-milled and air-classified field peas and horsebeans.

Tannins comprise a diverse group of polyphenolic compounds. High tannin content in food depresses growth when fed to chicks (Chang and Fuller 1964), increases fecal nitrogen when fed to rats (Glick and Joslyn 1970), and increases the resistance to bacterial degradation and in vitro digestibility of dry matter for ruminants (Ford 1978). The tannin content of beans depresses the apparent digestibility of casein for the rat (Mondragón and González 1978) and lowers the protein efficiency ratio (PER) of bean protein in rats (Ronnenkamp 1977). Chlorogenic acid when bound to casein slightly decreases in vitro digestibility and markedly depresses *Tetrahymena* growth, whereas unbound chlorogenic acid has little effect (Dryden et al 1977). Reports on the relationship between legume tannins or phytic acid and in vitro digestibility and between tannins or phytic acid and *Tetrahymena* growth are limited in number. Knowing how various protein and nonprotein constituents of food affect the *Tetrahymena* assays and, ideally, the PER assay as well is important. Such information is essential to determine whether the assay is a valid means of assessing the nutritive value of foods and feeds.

This study had two objectives. The first was to evaluate the effects of processing on the content and distribution of selected constituents in legumes. The second was to evaluate the effects of the constituents and of processing on in vitro digestibility and protein quality as measured by *T. pyriformis*.

MATERIALS AND METHODS

Lentils (*Lens culinaris*), green and yellow split peas (*Pisum sativum*), precooked powders, and sterilized flours were purchased in the spring of 1979 from a supplier in Moscow, ID, and Aurora white pea beans (*Phaseolus vulgaris*) from a supplier in Moses Lake, WA. The commodities were sampled, and the remaining portion was sent to the Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada, to be pin milled and air classified. The resulting protein and starch concentrates were stored in moisture-proof containers in the dark at ambient temperature and humidity until they were assayed. The relative humidity in the laboratory is generally low (35–50%), and the laboratory temperatures are maintained at 68 and 78° F during winter and summer, respectively.

Lentils, beans, and split peas were ground in a water-cooled micromill for 2 min. All other products were used in the forms in which they were received. All assays were done in triplicate.

The methods of analysis of the AOAC (1975) were followed to determine moisture, fat, ash, and total nitrogen by micro-Kjeldahl analysis. Phytic acid was determined as reported previously (Davis 1981), using a modification of the extraction procedure of Wheeler and Ferrel (1971) followed by measurement of the phytic acid phosphorus with the phosphomolybdate colorimetric method of Ward and Johnston (1962). Sodium phytate served as a standard for analysis. Total phosphorus was determined by the phosphomolybdate colorimetric procedure, using a hydrochloric

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[2]Assistant research professor, Food Research Center, Department of Bacteriology and Biochemistry, University of Idaho, Moscow 83843.

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acid solution of the ash (Ward and Johnston 1962).

The methods of tannin measurement are controversial. Maxson and Rooney (1972), for instance, suggested that because none of the methods they used could measure tannin content in clearly definable substances, a method should be selected to give results that are related to biologically significant differences in the quality of sorghum. Numerous methods are reported in the literature for measurement of tannins in different types of products. Because preliminary studies with these legume products using the Folin-Ciocalteu phenol reagent were not satisfactory, and because the vanillin-HCl method (Ma and Bliss 1978) resulted in little color formation, the tannin index method of Ford and Hewitt (1979) was selected. Ma and Bliss (1978) also found little tannin content in white beans using vanillin-HCl. For the analysis, triplicate 100-mg samples of the ground legumes were weighed into screw-capped 50-ml centrifuge tubes. To each tube was added 25 ml of 2% HCl in methanol. The tubes were capped and placed in a 48°C water bath for 4 hr (instead of the 16 hr in the original method). The tubes were shaken every 10 min. The extract was filtered, and the absorbance of the filtrate at 535 nm against a blank containing 2% HCl in methanol was determined and designated "tannin index." Owades et al (1958) reported that tannins could be measured at 272 nm in acidified methanol with catechin as a standard. Thus, the extracts from the tannin index were also measured at 272 nm, using catechin as a standard, and are reported as catechin equivalents.

Preliminary studies with the digestibility method showed no statistical difference between the method of Hsu et al (1977), using purified pancreatic enzymes, and the method of Marshall et al (1979), using an activated pancreatin preparation made from porcine pancreas, when the regression equation for plant products was used. Economics influenced the decision to use the crude pancreatin preparation in this study.

Following the determination of digestibility, the pH was adjusted to 8 and the hydrolysates diluted to provide 0.3 mg of N per milligram. The early samples were filtered through fritted glass of medium porosity. However, the filtration was very slow, and cleaning the funnels was difficult. Centrifugation for 15 min at 10,000 rpm and 4°C proved to be more satisfactory. The clear filtrate or supernate was frozen until used for the *Tetrahymena* relative nutritive value (T-RNV) assay.

The *T. pyriformis* culture was alternated weekly between proteose peptone and yeast extract to prevent adaptation of the organism to one medium (Dryden et al 1977). The inoculum was prepared by adding 2 ml of a proteose peptone culture three to four days old to 10 ml of sterile distilled water (Sutton 1978).

The test culture medium, that of Baker et al (1978) minus the asparagine, has been designated "modified Baker's medium." The change was made in accordance with the report of Evans (1978) that *Tetrahymena* needs an essential-nonessential amino acids ratio of about 2:1 for maximum growth. We felt that the incorporation of nonessential amino acid nitrogen from the asparagine would be especially prejudicial toward lower quality proteins. Furthermore, the concept of a test medium is that it should supply all essential nutrients except the one being tested, amino acid nitrogen in this case. The modified Baker's medium was prepared by suspending 2.7 g of mix in 100 ml of glass-distilled water and heating to a boil in a microwave oven. For each sample, three 25-ml micro-Fernbach flasks containing 2.5 ml of medium were prepared, and 2.5 ml of the hydrolysate was added. The flasks, stoppered with foam rubber plugs, were sterilized by being heated in an autoclave for 15 min at 15 lb. Inoculum (0.2 ml) was aseptically added to each flask and the samples incubated at 26 ± 1°C for three days. One milliliter of the contents of each flask was added to 1 ml of isotonic formalin (Stott et al 1963) to stop growth and preserve the organisms. A direct microscopic count, using a hemacytometer with a modified Neubauer ruling, was used to enumerate the growth of the organisms. The T-RNV was calculated as:

$$\text{T-RNV} = \frac{\text{number of organisms per milliliter of sample}}{\text{number of organisms per milliliter of casein}} \times 100.$$

Statistical analysis was run by computer, using the general linear models procedure (Harvey 1975) and the stepwise regression procedure to determine the maximum R^2 for each dependent variable (Barr 1979). The dependent variables were digestibility,

TABLE I
Proximate Composition (%) and Phosphorus Content (%) of Green and Yellow Split Peas, Lentils, and Aurora Beans in the Forms of Powder, Flour, and Protein and Starch Concentrates[a]

	H_2O	Fat	Ash	Protein[b]	Phosphorus
Green Peas					
Split	7.59 ± 0.05	0.80 ± 0.02	2.70 ± 0.10	34.34 ± 0.13	0.354 ± 0.01
Powder	9.25 ± 0.06	1.88 ± 0.07	2.65 ± 0.11	24.66 ± 0.16	0.389 ± 0.09
Flour	9.75 ± 0.06	1.16 ± 0.05	2.82 ± 0.03	24.61 ± 0.30	0.434 ± 0.11
Concentrate					
Protein	6.17 ± 0.12	2.05 ± 0.04	5.17 ± 0.07	63.53 ± 0.27	0.691 ± 0.35
Starch	6.87 ± 0.04	0.23 ± 0.00	1.13 ± 0.06	10.03 ± 0.02	0.178 ± 0.05
Yellow peas					
Split	7.23 ± 0.06	0.54 ± 0.08	2.52 ± 0.11	27.70 ± 1.00	0.405 ± 0.49
Powder	8.95 ± 0.06	1.70 ± 0.05	2.65 ± 0.04	23.40 ± 1.38	0.467 ± 0.01
Flour	10.07 ± 0.02	0.93 ± 0.04	2.77 ± 0.12	24.97 ± 0.64	0.467 ± 0.07
Concentrate					
Protein	5.92 ± 0.02	1.91 ± 0.15	5.04 ± 0.03	59.46 ± 0.87	1.047 ± 0.35
Starch	6.76 ± 0.02	0.25 ± 0.09	1.38 ± 0.01	11.10 ± 0.60	0.231 ± 0.05
Lentils					
Whole	7.59 ± 0.10	0.90 ± 0.01	2.87 ± 0.25	29.07 ± 0.10	0.565 ± 0.32
Powder	9.00 ± 0.04	1.62 ± 0.08	2.70 ± 0.06	28.19 ± 0.20	0.507 ± 0.07
Concentrate					
Protein	5.61 ± 0.02	2.33 ± 0.19	5.51 ± 0.08	63.59 ± 1.42	1.168 ± 0.05
Starch	6.48 ± 0.02	0.68 ± 0.02	1.87 ± 0.09	16.41 ± 0.04	0.368 ± 0.29
White Bean					
Whole	10.72 ± 0.54	1.55 ± 0.03	4.29 ± 0.05	25.58 ± 0.05	0.561 ± 0.08
Concentrate					
Protein	5.92 ± 0.03	3.20 ± 0.21	7.46 ± 0.12	53.14 ± 1.97	1.183 ± 0.02
Starch	8.10 ± 0.11	2.02 ± 0.12	2.74 ± 0.13	16.04 ± 0.56	0.303 ± 0.33

[a] All data are reported on a moisture free basis and are presented as mean ± standard deviation of three replications.
[b] Protein was calculated as N × 6.25.

relative nutritive value, and protein content. Independent variables were phytic acid, phosphorus, tannin index, and catechin equivalents. Protein was also included as an independent variable for digestibility and relative nutritive value. The general linear models procedure was used to test variety and treatment effects and the stepwise regression procedure to test multiple correlations. Sample correlations comparing each variable with all other variables were also run.

RESULTS AND DISCUSSION

Analytical results for the various constituents on a moisture free basis are presented in Tables I and II. Digestibility and T-RNV are expressed on an isonitrogenous basis. Average values for all constituents are listed by variety and by treatment in Table III.

Variety had a significant influence on moisture, fat, ash, tannin index, phosphorus, T-RNV, and digestibility. Aurora products had more moisture than did green and yellow pea or lentil products, with an average of 8.25, 7.92, 7.78, and 7.17%, respectively (Table III). Fat content was highest in Aurora and lowest in yellow pea products, with an average of 2.26 and 1.07%, respectively. Ash was highest in Aurora and lowest in the green and yellow pea products, with an everage of 4.83, 2.90, and 2.87%, respectively. Tannin index was approximately 10 times greater in lentil products (2.458) than in yellow pea and Aurora products (0.251). Lentil products also had the highest content of catechin equivalents (0.396%) and yellow pea products the lowest (0.277%). The lowest digestibility was

TABLE II
Phytic Acid, Tannin Index, Digestibility, and *Tetrahymena* Relative Nutritive Value (T-RNV) of Green and Yellow Split Peas, Lentils, and Aurora Beans in the Form of Powders, Flours, and Protein and Starch Concentrates[a]

	Phytic Acid[b] (mg/g)	Tannin Index[b]	Catechin Equivalents[b] (%)	Digestibility[c] (%)	T-RNV[c] (%)
Green Peas					
Split	5.1 ± 0.30	0.023 ± 0.001	0.28 ± 0.01	84 ± 1.24	50 ± 2.41
Powder	6.7 ± 0.72	0.036 ± 0.006	0.26 ± 0.01	87 ± 0.46	40 ± 4.06
Flour	7.2 ± 0.35	0.068 ± 0.006	0.25 ± 0.02	82 ± 0.45	45 ± 3.45
Concentrate					
Protein	6.1 ± 0.39	0.041 ± 0.003	0.53 ± 0.05	84 ± 2.03	48 ± 0.01
Starch	0.1 ± 0.01	0.014 ± 0.001	0.12 ± 0.01	82 ± 0.62	62 ± 3.21
Yellow Peas					
Split	5.4 ± 0.13	0.025 ± 0.002	0.29 ± 0.03	83 ± 0.82	29 ± 4.48
Powder	8.0 ± 0.07	0.021 ± 0.002	0.22 ± 0.01	88 ± 0.82	33 ± 4.73
Flour	7.5 ± 0.42	0.030 ± 0.002	0.29 ± 0.01	83 ± 0.76	44 ± 3.69
Concentrate					
Protein	5.8 ± 0.11	0.037 ± 0.001	0.41 ± 0.01	83 ± 0.23	31 ± 2.51
Starch	0.2 ± 0.07	0.011 ± 0.002	0.12 ± 0.01	81 ± 1.27	25 ± 3.23
Lentils					
Whole	8.1 ± 0.31	0.270 ± 0.037	0.49 ± 0.02	80 ± 0.67	42 ± 4.14
Powder	7.2 ± 0.20	0.209 ± 0.001	0.39 ± 0.02	84 ± 0.56	30 ± 1.70
Concentrate					
Protein	10.2 ± 0.30	0.159 ± 0.006	0.38 ± 0.00	83 ± 0.42	55 ± 3.69
Starch	5.1 ± 0.02	0.346 ± 0.055	0.337 ± 0.058	79 ± 0.27	37 ± 0.82
White Bean					
Whole	11.0 ± 0.64	0.030 ± 0.002	0.34 ± 0.02	77 ± 4.34	74 ± 5.31
Concentrate					
Protein	14.1 ± 0.06	0.030 ± 0.003	0.32 ± 0.00	78 ± 2.19	70 ± 4.02
Starch	6.0 ± 0.00	0.016 ± 0.002	0.20 ± 0.02	71 ± 0.78	64 ± 2.72
Casein	...	...	...	92 ± 2.50	100

[a] Data reported as mean ± SD of three replications.
[b] Moisture free basis.
[c] Isonitrogenous basis.

TABLE III
Proximate Composition, Phosphorus, Phytic Acid, Tannin Index, Catechin Equivalents, Digestibility, and T-RNV[a] Average Values by Variety and by Treatment[b]

Mean	H_2O (%)	Fat (%)	Ash (%)	Protein (%)	Phytic Acid (mg/100 g)	Phosphorus (%)	Tannin Index Units	Catechin Equivalent (%)	Digestibility (%)	T-RNV (%)
Variety										
Green Pea	7.92	1.23	2.90	31.41	502.	0.409	0.365	0.289	83.73	49.44
Yellow Pea	7.78	1.07	2.87	29.33	536.	0.524	0.251	0.277	83.46	32.53
Lentil	7.17	1.38	3.24	34.31	763.	0.652	2.458	0.396	81.59	41.15
Aurora	8.25	2.26	4.83	31.59	1036.	0.682	0.251	0.287	75.46	69.00
Treatment										
Intact	8.28	0.95	3.10	29.15	739.	0.471	0.869	0.351	80.83	48.73
Powder	9.06	1.73	2.67	25.41	727.	0.454	0.887	0.288	86.36	34.77
Flour	9.91	1.04	2.80	24.79	732.	0.450	0.492	0.271	83.14	44.24
Concentrate										
Protein	5.90	2.37	5.80	58.93	904.	1.022	0.667	0.411	82.25	50.90
Starch	7.05	0.79	1.78	13.39	286.	0.270	0.968	0.198	78.26	47.12

[a] *Tetrahymena*-relative nutritive value.
[b] Averages are reported on a moisture free basis, except for digestibility and T-RNV, which are reported on an isonitrogenous basis.

observed in Aurora (75.46%). Lentil and the pea products had a range from 81.59 to 83.73% for digestibility. Relative nutritive value was highest in Aurora products (69%) and lowest in yellow pea (32.53%).

Treatment effects were significant for moisture, fat, ash, protein, phosphorus, phytic acid, catechin equivalents, digestibility, and T-RNV. Moisture was lowest in the protein concentrates and highest in the sterilized flours, with an average of 5.90 and 9.91%, respectively (Table III). Fat was lowest in the starch concentrates and highest in the protein concentrates, with an average of 0.79 and 2.37%, respectively. Other constituents that were partitioned into the protein concentrates include ash (5.80%), protein (58.93%), phytic acid (904 mg/100 g), phosphorus (1.022%), catechin equivalents (0.411%), and the pigment responsible for the tannin index (0.667 tannin index units). Additionally, the T-RNV was highest in the protein concentrates. Only the tannin index was increased in the starch concentrate. An association between phosphorus, phytic acid, and protein is suggested by the fact that all three were partitioned into the protein concentrate and is substantiated by simple correlations (Table IV). A similar partitioning during pin milling and air classification of horsebeans and field beans was reported by Vose et al (1976). Bourdillon (1951) showed that the phytate of bean seed is present as a complex with the protein.

Phosphorus was partitioned into the protein fraction during the pin milling and air classification to a level (1.022%) approximately double that (0.471%) of the intact legumes. Phytic acid, the major storage form of phosphorus, was increased in the powder and flour of the split peas but not in that of the lentils. In all cases, the phytic acid was increased in the protein concentrate (904 mg/100 g) and reduced in the starch concentrate (286 mg/100 g) as compared to the level in the intact legume (739 mg/100 g).

The catechin equivalents assay, one measure of tannin content, was influenced significantly be treatment. The catechin equivalents were higher in the protein concentrate (0.411%) than in the intact legume (0.351%). In all cases, the starch concentrate (0.198%) had a lower level of catechin equivalents than did the intact legumes. Although the tannin index was not significantly different by treatment, some important changes were noted, especially in the lentils. The seed coat of the lentil, which is highly pigmented, was determined by visual examination to be concentrated in the starch fraction. Likewise, an increased tannin index was observed in the starch fraction. In all other legumes, the protein concentrate had a higher tannin index than did the starch concentrate. This variety-treatment interaction probably resulted in nonsignificance of the tannin index. The differences between tannin index and catechin equivalents also demonstrates that the two methods measure different aspects of tannin, or phenolic content.

Broadhurst and Jones (1978) reported that flavanols react with vanillin-HCl to produce a chromophore with an absorption maximum at 500 nm and that anthocyanidins interfered with the measurement of the vanillin-HCl color. Ma and Bliss (1978) found maxima at 500 and 535 nm for bean tannins and at 488 nm for catechin. Ford (1978) showed that methanol-HCl extracts of legumes contain anthocyanidins that give a magenta color, one of which has an absorption maximum at 530 nm. In the methanol-HCl extracts of lentil products in this study, we observed a deep magenta color with an absorption maximum at 532 nm. This pigment resulted in a higher tannin index for lentil and lentil products than for the other samples. Ma and Bliss (1978) reported that tannins were localized in the testa of common beans. Thus, the pigments of the lentils measured by the tannin index appear to be anthocyanidins extracted from the seed coat.

The stepwise maximum R multiple correlation analyses showed the protein content to be significantly correlated with phytic acid, phosphorus, tannin index, and catechin equivalents (Table V). The coefficient of determination (R^2) was 0.93 for a model incorporating the four variables that met the 0.5 level of significance for the partial correlation coefficient, which was used as the limit for entry into the model. Phytic acid and tannin index were negatively correlated with protein content (Table V), whereas phosphorus and catechin equivalents were positively correlated.

Simple correlations showed protein to have a highly significant negative association with moisture content and highly significant positive associations with fat, ash, phytic acid, phosphorus, and catechin equivalents (Table IV). The maximum R analysis resulted in a four-variable model for predicting protein content that included phytic acid, phosphorus, tannin index, and catechin equivalents (Table V). The type II sum of squares, obtained using the interaction as an error term, reduces the "error" degrees of freedom and increases the size of the denominator used in calculating the F statistic. The overall effect is to make a more conservative evaluation of the statistical significance of the variables. In this particular case, phytic acid went from a positive simple correlation to a negative multiple correlation. The apparent

TABLE IV
Summary of Simple Correlations with r ≥0.5 or ≤−0.5

X Variable	Y Variable	Correlation Coefficient (r)[a]
Variety	Phytic acid	0.5681
	Digestibility	−0.6600
Treatment	H_2O	−0.5287
H_2O	Treatment	−0.5287
	Protein	−0.5006*
Fat	Ash	0.8505
	Protein	0.6769
	Phytic acid	0.7620
	Phosphorus	0.7632
Ash	Fat	0.8505
	Protein	0.8657
	Phytic acid	0.7711
	Phosphorus	0.9238
	Catechin equivalents	0.5389
Protein	H_2O	−0.5006*
	Fat	0.6769
	Ash	0.8657
	Phytic acid	0.5103
Phytic acid	Variety	0.5681
	Fat	0.7620
	Ash	0.7711
	Protein	0.5103
	Phosphorus	0.6956
Phosphorus	Fat	0.7632
	Ash	0.9238
	Protein	0.8846
	Phytic acid	0.6956
	Catechin equivalents	0.5532
Catechin equivalents	Ash	0.5389
	Protein	0.6922
	Phosphorus	0.5532
Digestibility	Variety	−0.6600
	Relative nutrient value	−0.5122

[a] $P >$ (r) = 0.0001, except * = 0.0002.

TABLE V
Analysis of Variance for the Maximum R^2 Multiple Regression of Protein Content on Phytic Acid, Phosphorus, Tannin Index, and Catechin Equivalents[a]

Source	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F	$P > F$
Regression	4	13,392.767	3,348.044	150.79	0.0001
Error	45	999.144	22.203		
Total	49	14,391.321			

Source	B Value[b]	Type II Sum of Squares	Calculated F	$P > F$
Phytic acid	−13.340	475.116	21.40	0.0001
Phosphorus	46.175	4,024.619	181.26	0.0001
Tannin index	− 0.004710	839.360	37.60	0.0001
Catechin equivalents	− 0.074321	1,723.207	77.60	0.0001

[a] $R^2 = 0.9306$.
[b] An indicator of the slope contributed by each variable in the model.

reason seems to be the interaction between protein and phytic acid in protein concentrates and in starch concentrates—peas, beans, and lentils don't react in the same way, or to the same degree, to the treatments.

Significant differences were found in protein digestibility due to treatment effects (Tables II and III). Digestibility of the protein in the powders (86.36%) increased approximately 6% as compared to that of the intact legumes (80.83%). An increase would be expected if proteinaceous protease inhibitors were destroyed by a cooking process. The lowest digestibilities were observed in the starch concentrates (78.26%). We do not know why the protein associated with the starch should be less digestible than protein in the intact legume or in the protein concentrate (82.25%). Vose et al (1976) observed that the protein of the starch fraction was easily solubilized and removed from the starch, so competitive binding between the proteolytic enzyme and starch with the protein seems unlikely. The protein concentrates were slightly more digestible than the intact legume, again suggesting that proteinaceous protease inhibitors may be associated with the starch fraction.

Only five independent variables met the 0.5 level of significance for the partial correlation coefficient that we required for entry into the stepwise maximum R analysis for digestibility. The best model for predicting digestibility was a three-variable model that included moisture, protein, and ash (Table VI). Moisture and protein were positively correlated with digestibility, and ash was negatively correlated. Although this three-variable model is highly significant, it accounts for only 40% of the variability observed in the digestibility assay. Protease inhibitors, amino acid composition, and perhaps forms of polyphenolic compounds not measured by the methods of this study could all have major effects on digestibility. Silicate, either as ash or as soluble silicate, can depress the in vivo and in vitro digestibility of dry matter in forage crops (Smith and Urquhart 1975, Smith et al 1971, Van Soest and Jones 1968). Thus, the very significant depression of in vitro digestibility associated with increased ash content may reflect the effect of silicate in the ash, as has been observed in ruminants (Smith et al 1971, Van Soest and Jones 1968).

Treatment effects were not significant on T-RNV because of the large variety-treatment interaction. The lowest T-RNVs were observed in the precooked powder form of green peas and lentils (Table II). A depressed T-RNV has previously been observed for *Tetrahymena* in cooked versus raw beans either with pepsin predigestion, pancreatin predigestion, or no predigestion.[3] Achinewhu and Hewitt (1979) reported that heat treatment of legumes decreased the digestibility of all amino acids and the availability of lysine and methionine when measured using ileal digestibility in rats. Almas and Bendor (1980) showed that lysine is destroyed by cooking legumes. Autoclaving for 30 min or boiling for 2 hr resulted in losses of 9–12% of the available lysine, with greater losses in red beans than in white. Loss in reducing sugar was also reported to be correlated with the losses of available lysine.

Because lentils contain large amounts of tannins, water-soluble tannins may have migrated from the testa into the cotyledons during the cooking process and crosslinked the protein by reacting with lysine or methionine or may have chelated essential minerals and made them unavailable to the organism (Sosulski 1979). Water-soluble tannins may also be ingested by the organism and be toxic. The organism has a slight ability to detoxify phenol in the medium (Schultz and Dumont 1977). In mammalian systems, the pathway involves a methylation, and, if the organism detoxifies tannins in this manner, that would further stress the limited methionine content of the legumes. That the tannins in lentils, as measured by the tannin index, reduce the T-RNV is shown in the pin-milled and air-classified products. The starch fraction contains the highest tannin index and the next to lowest T-RNV, whereas the protein fraction contains the lowest tannin index and the highest T-RNV. The increased tannin index in the lentil starch fraction was a result of the partitioning of the heavier testa fragments into the starch. Dryden and Satterlee (1978) reported that chlorogenic acid depressed *Tetrahymena* growth when bound to casein but did not do so as free chlorogenic acid in the medium. This further suggests that seed coat tannins depress T-RNV by binding to proteins. Ronnenkamp (1977) observed a depressed PER in the rat when colored testa was added to a white bean diet. Knowing whether the depressed growth of *Tetrahymena* resulting from tannins would parallel the depressed growth of rats as observed by Mondragón and Gonzáles (1978) and by Ronnenkamp (1977) would be useful. The tannin content of yellow peas, green peas, and Aurora beans, as measured by the methods reported in this article, is not high enough to exert an effect on the organism. Ford and Hewitt (1979) reported that only varieties of legumes with colored flowers had a significant tannin index. We do not know what factor in yellow peas is inhibitory to the growth of the organisms. However, the improved T-RNV in heat-treated forms (powder and flour) is suggestive of heat-labile components, possibly protease inhibitors.

Stepwise maximum R analysis (Table VII) showed that 46.98% of the variability observed in T-RNV could be predicted from a model containing ash, protein, and phosphorus as independent variables. Protein and phosphorus were negatively correlated with T-RNV. The addition of moisture, fat, phytic acid, tannin index, and catechin equivalents only increased the coefficient of determination to 52%. Of those additional variables, fat, phytic acid, and catechin equivalents all had a negative effect, although they were not significant. The fact that tannin index had a positive correlation with T-RNV and catechin equivalents a negative correlation further demonstrates that the two methods measure different components. A portion (47–52%) of the variability in T-RNV is associated with changes in protein associated with components not usually considered when evaluating protein nutritive quality. A confusing finding is that digestibility and T-RNV are negatively correlated. Fat was negatively correlated with T-RNV. Fat was not present in the samples in sufficient quantity to form a barrier to gas exchange as reported by Evans et al (1978). Thus, some ether-extractable constituent that inhibits growth of the organism must be present in legumes. Organisms grown on

TABLE VI
Analysis of Variance for the Maximum R^2 Multiple Regression of Digestibility on Moisture, Ash, and Protein[a]

Source	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F	$P > F$
Regression	3	321.604	107.2014	10.06	0.0001
Error	46	490.2182	10.6569		
Total	49	811.8223			

Source	B Value[b]	Type II Sum of Squares	Calculated F	$P > F$
Moisture	0.8348	59.0656	5.54	0.0229
Ash	−2.9147	263.3556	24.71	0.0001
Protein	0.3288	316.9613	29.74	0.0001

[a] $R^2 = 0.3962$.
[b] An indicator of the slope contributed by each variable in the model.

TABLE VII
Analysis of Variance for Maximum R^2 Multiple Regression of *Tetrahymena* Relative Nutritive Value on Ash, Protein, and Phosphorus as Independent Variables[a]

Source	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F	$P > F$
Regression	3	4,993.17	166,439	13.29	0.0001
Error	46	5,634.33	12,521		
Total	48				

Source	B Value[b]	Type II Sum of Squares	Calculated F	$P > F$
Ash	15.774	4,372.89	34.92	0.0001
Protein	−00.603	1,080.10	8.63	0.0052
Phosphorus	−38.41	780.77	6.24	0.0162

[a] $R^2 = 0.4698$.
[b] An indicator of the slope contributed by each variable in the model.

[3] Unpublished work by K. R. Davis at Washington State University (1976–1978) and at the University of Idaho (1978–1981).

green or yellow pea flour were very large—three to four times as long as organisms grown on casein. Likewise, those organisms grown on green pea starch were large but also green in color. Perhaps if the total cell volume of *Tetrahymena* grown on these pea flours and green pea starch were used to calculate T-RNV, these legumes would equal or exceed the relative nutritive value of other legumes.

T-RNVs ranged from 30% for lentil powder to 74% for Aurora white bean as compared to 100% for casein (Table II). Values found in the literature for similar products using *Tetrahymena* as a test organism included 91% for green peas (Helms and Rølle 1970), 34% for First and Best peas (Evans and Bandamer 1967), 14% for Sanilac beans (Evans and Bandamer 1967), 14.9–18.9% for lentils (calculated from McCurdy et al 1978), and 46.0–54.5% for field peas (Maciejewicz-Rys and Antoniewicz 1978). White beans (raw) tested with the method of McCurdy et al (1978) had T-RNVs of 44 and 57%, whereas two other varieties of white beans tested with our method had 55 and 60% T-RNVs (Davis[3]). Ford and Hewett (1979) reported relative nutritive values of 58 and 71% in white flowered beans and 57–68% in colored flowered varieties of beans using *Streptococcus zymogenes* as a test organism.

Compared to the other legumes in this study, the Aurora products were of high protein quality as measured by the T-RNV and are deserving of further evaluation. The improved protein quality in the green and yellow starch concentrates should be investigated. A recent report by Patel et al (1980) demonstrated that the methionine was concentrated in the starch fraction of air-classified navy bean flour. A higher level of methionine would be conducive to better growth of *Tetrahymena* and might explain the improvement noted in the pea starch fractions in this study, although an improvement was not seen in Aurora bean starch.

Several questions raised by this study need further research. Do *Tetrahymena* and the rat (or human) respond in the same way to tannins, ash content, and fat content of the samples? How well do the T-RNVs correlate with essential amino acid content? What is the significance of the inverse relationship between in vitro digestibility and T-RNV? Which constituents of the fat and ash, if any, contribute to the depressed T-RNV? Which polyphenolic constituents depress in vitro digestibility? Which, if any, of the polyphenolic constituents depress T-RNV? Is additional methionine protective against the effects of tannins? What is the difference between green and yellow peas? Do rats and *Tetrahymena* respond in the same manner to green and yellow peas? Is total cell volume a better measure of protein quality than number of cells?

SUMMARY

This study has shown that the in vitro digestibility and T-RNV assays are sensitive enough to detect differences in legumes resulting from processing and from species of legume. It has shown by multiple regression analysis that moisture, ash, and protein are significantly associated with digestibility and that ash, protein, and phosphorus are significantly associated with T-RNV. Furthermore, in all cases, less than 50% of the variability in T-RNV and digestibility were accounted for by all of the variables tested. Thus, amino acid composition, the presence of inhibitors, and other unknown factors must account for more than 50% of the variability observed in T-RNV and digestibility. However, a model relating T-RNV with rat PER may need to include these factors. This study has shown that a single simple food is a very complex mixture and we don't know the effects of the individual constituents nor the effects of interactions of the constituents on protein quality as measured by digestibility or T-RNV.

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