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Chemical and nutritional quality changes in germinating seeds of *Cajanus cajan* L.

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Abstract

Changes in the chemical constituents and nutritive quality of germinating seeds of pigeon pea, *Cajanus cajan* L. were studied in seeds that were germinated for 0, 1, 2, 3, 4 and 5 days. The differently germinated seeds were analysed for proximate composition, mineral elements, structural carbohydrates, calorific value, nutritive and non-nutritive matter and certain antinutritional factors. Germination significantly altered the nutrient composition of the seed, causing marked increase in calorific value. Crude protein, soluble carbohydrate, cellular and organic cellular contents, cellulose, lignin, non-nutritive matter, total oxalate and phytic acid contents of the seed were negatively correlated with germination, whereas the reverse was the case with the seed's contents of fat, crude fibre, total ash, soluble ash, acid-insoluble ash, cell wall carbohydrate, hemicellulose, iron, manganese, calcium, magnesium, copper, phosphorus, food energy, digestible energy, tannins, total phenolics and trypsin inhibitory activity. It was concluded that the increased contents of tannins, total phenolics and trypsin inhibitory activity of the seed during the progressive germination might limit its nutritive quality.

Keywords: Pigeon pea; Germination; Chemical composition; Antinutritional factor; Nutritive quality evaluation

1. Introduction

Legume seeds offer protein cost advantages over animal protein in human nutrition particularly in countries, including Nigeria, where animal protein is expensive and is in inadequate supply (Nestec, 1987). Unfortunately, legume seeds contain antinutritional factors, such as enzyme inhibitors, phytates, oxalates, saponins and polyphenolic compounds, all of which, limit their utilisation (Liener, 1980). However, remarkable improvements in the nutritive value of legume seeds have been achieved by dehulling, heat treatment, partial hydrolysis by proteolytic enzymes and germination (Bansal, Dhindsa, & Batra, 1988; Barroga, Laurena, & Mendons, 1985; Batra, Vasishta, & Dhindsa, 1986; Elemo, Egun, Oladimeji, Nwadei, & Adewunmi, 1998; Haider, 1981; Shastry & John, 1991). The present study reports changes in the chemical composition and nutritional quality of the seed of *Cajanus cajan* as affected by germination.

As part of the efforts made to solve the problem of low protein intake in Nigeria, nutritionists have advocated increased consumption of food legumes, such as *Vigna unguiculata* and *Glycine max* in the campaign. Yet unexploited is the utility of the seeds of *C. cajan,* whose cultivation is well supported by the soil and prevailing climatic conditions of the western region of Nigeria. Indeed, in this geographical zone of the country, the seeds are boiled and eaten by natives (Kay, 1979; Oyenuga, 1968). Like other legumes, the need for dealing with the anti-nutritional principles in *C. cajan* has been noted in earlier studies (Batra et al., 1986).

2. Materials and methods

2.1. Sample collection and germination

A bulk of 3000 healthy and clean seeds of pigeon pea, *C. cajan* L. cv IITA 8860 used in this study was obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria. The bulk was divided into six equal portions. The first portion was reserved as control (ungerminated seeds) while the remaining five portions were allowed to germinate for 1, 2, 3, 4 or 5

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days. Each of the five portions that were allowed to germinate was further subdivided into five equal groups so that germination trials had five replicates. Prior to germination, seeds were soaked at room temperature in deionised water for 18 h. Germination was done in sterile petri-dishes lined with wet cotton wool for the respective number of days. At the end of the respective germination periods, replicated samples were pooled, freeze-dried, ground separately to pass through a 40-mm mesh sieve in preparation for subsequent chemical analysis. The control group (i.e. un-geminated seeds) were freeze-dried and ground, sieved and kept for chemical analysis just like the germinated seeds.

2.2. Analytical procedures

Samples of un-germinated and germinated seeds were analysed for total nitrogen, ether extract, crude fibre and ash (AOAC, 1980). Crude protein was calculated by multiplying the per cent Kjeldahl nitrogen by the factor 6.25. Nitrogen free extract (NFE) was estimated by difference. Total ash was fractionated into soluble ash and acid insoluble ash, using the method of Egan, Kirk, and Sawyer (1981). The energy content was determined by multiplying the percentages of crude protein, crude fat and NFE by the factors of 4, 9, and 4, respectively (Osborne & Voogt, 1978).

Calcium, magnesium, manganese, iron and copper were analysed by an atomic absorption spectrophotometric method (Perkin-Elmer Inc., 1973). Phosphorus content was determined colorimetrically using the phosphovanado molybdate method of AOAC (1980). Phytic acid (Wheeler & Ferrel, 1971), total oxalate (Krishna & Ranjhan, 1980), and trypsin inhibitory activity (Kakade, Simons, & Liener, 1969) were determined in all samples. Tannin was estimated using catechin as the reference standard (Burns, 1971), while total phenolics were estimated using chlorogenic acid as standard (Swain and Hillis, 1959).

Cell wall carbohydrate (CWC), cellulose, hemicellulose, cellular content (CC), organic cellular content (OCC), soluble carbohydrate (SC), non-nutritive matter (NNM) and lignin contents of all samples were determined (Fonnesbeck, 1976). Digestible energy values of samples for different laboratory animals were estimated by fitting data from chemical analyses into prediction equations described by Fonnesbeck (1976).

2.3. Statistical analysis

Data obtained from the study were subjected to statistical analysis in accordance with the procedures of Gomez and Gomez (1976). Significantly different treatment means were compared by the method of Duncan (1955).

3. Results and discussion

Results presented in Table 1 indicated remarkable changes in proximate composition and food energy values of pigeon pea due to germination. As germination progressed, crude protein and nitrogen free extractives (crude carbohydrate) decreased gradually, whereas fat, crude fibre, ash and food energy increased. While crude protein and carbohydrate were significantly and negatively correlated with period of germination, the reverse was the case for fat, crude fibre, ash and food energy (Table 2). Estimated regression equations, describing the responses of the changes in the nutrients to germination, are given in Table 2. The decline in the contents of crude protein and carbohydrate of the pigeon pea seed during germination are in agreement with observations made on germinating *Phaseolus vulgaris* (Nielsen & Liener, 1984), *V. unguiculata* (Ologhobo & Fetuga, 1986) and Dolichos*lablab* (Shastry & John, 1991). The authors attributed the reduction of both nutrients to their utilisation in the germination process. Reduction of pigeon pea seed's storage protein and carbohydrate resulted in concomitant increase in the other nutrients that is, fat, crude fibre and ash. The greatest increase was found in fat contents and, because

Table 1
Proximate composition and energy value of *Cajanus cajan* seeds as affected by germination[a]

Germination (day)	Crude protein (%)	Fat (%)	Nitrogen free extractives (%)	Crude Fibre (%)	Ash (%)	Energy (Kcal/100 g)
0	21.9a[b]	2.70d	62.6a	8.30	4.60c	362c
1	20.4a	3.58d	62.5a	8.61	4.86c	364bc
2	18.8b	4.99c	61.5b	8.80	5.94b	366b
3	17.3b	5.35c	61.5b	9.36	6.55b	363c
4	16.5c	6.63b	60.0c	9.26	7.56a	366b
5	15.3d	7.98a	59.5c	9.46	7.82a	370.78a
±S.E.M.[c]	1.02	0.790	0.518	0.190	0.548	1.27

[a] Means of four replicate determinations expressed on dry weight basis.
[b] Mean values in a column denoted by different letters differ significantly at $P \leqslant 0.05$.
[c] S.E.M., standard error of the mean.

Table 2
Regression equations showing relationship between parameter and germination period

Parameter	Regression equation[a]	R^2	r
Crude protein (%)	$Y = 0.0771x^2 - 1.8651x + 23.768$	0.996	−0.994
Fat (%)	$Y = 0.0327x^2 + 0.7972x + 1.919$	0.987	0.992
Nitrogen free extractives (%)	$Y = -0.0745x^2 - 0.1302x + 62.845$	0.949	−0.961
Crude fibre (%)	$Y = -0.0305x^2 + 0.4512x + 7.849$	0.943	0.954
Total ash (%)	$Y = -0.005x^2 + 0.7439x + 3.694$	0.975	0.988
Food energy (kcal/100 g)	$Y = 0.4108x^3 - 2.7764x^2 + 5.432x + 361.75$	0.934	0.798
Cellular content (%)	$Y = -0.2374x + 91.629$	0.910	−0.954
Organic cellular content (%)	$Y = -0.8771x + 87.713$	0.983	−0.991
Soluble ash (%)	$Y = 0.6397x + 3.9157$	0.973	0.987
Soluble carbohydrate (%)	$Y = -0.0834x^2 - 0.2065x + 63.334$	0.896	−0.929
Acid insoluble ash (%)	$Y = 0.0004x^2 + 0.0666x + 0.468$	0.965	0.982
Cell wall carbohydrate (%)	$Y = 0.0313x^2 - 0.0067x + 8.723$	0.997	0.976
Cellulose (%)	$Y = -0.418x + 4.868$	0.954	−0.989
Hemicellulose (%)	$Y = 0.63x + 3.5633$	0.955	0.992
Lignin (%)	$Y = -0.0016x^2 - 0.135x + 1.637$	0.975	−0.987
Non-nutritive matter (%)	$Y = -0.0012x^2 - 0.0684x + 2.105$	0.958	−0.977
Fe (mg/100 g)	$Y = 0.1205x^2 + 0.0214x + 5.277$	0.990	0.975
Mn (mg/100 g)	$Y = 0.0024x^3 - 0.031x^2 + 0.1523x + 2.8333$	0.579	0.735
Ca (mg/100 g)	$Y = -1.7787x^2 + 19.498x + 118.41$	0.911	0.895
Mg (mg/100 g)	$Y = 0.4161x^2 + 1.3361x + 85.99$	0.964	0.972
Cu (mg/100 g)	$Y = 0.0021x^3 - 0.0275x^2 + 0.1175x + 0.9633$	0.894	0.801
P (mg/100 g)	$Y = 0.26x^2 + 5.4679x + 281.7$	0.953	0.975
Total Oxalate (mg/100 g)	$Y = -2.784x + 18.124$	0.985	−0.993
Tannins (mg/100 g)	$Y = 0.3616x^2 - 2.0198x + 3.565$	0.917	0.666
Total phenolics (μg/100 g)	$Y = -0.5186x^2 + 24.599x - 7.336$	0.962	0.980
Trypsin inhibitory activity[b]	$Y = 1.128x^2 - 3.5025x + 18.377$	0.950	0.912
Phytic Acid (mg/100 g)	$Y = -122.38x + 955.27$	0.984	−0.992
Mean Digestible energy (kcal/100 g DM)	$Y = 0.0547x^3 - 0.6315x^2 + 3.7698x + 363.75$	0.948	0.959

[a] Y, Parameter; x, germination period (days).
[b] Expressed as units of enzyme activity inhibited per mg protein.

fat contains about twice the food energy values of protein and carbohydrate (Osborne & Voogt, 1978), the increase in food energy value of the germinating seeds might be attributed to the increase in fat content.

Contents of the mineral elements and antinutritional factor in the seeds of pigeon pea as affected by germination, are shown Tables 3 and 4. All the mineral elements and the antinutritional factors estimated in this study, excepting total oxalate and phytic acid, were positively correlated with duration of germination (Table 2). Also, estimated regression equations, describing the relationships between the constituents and period of germination, are given. All the mineral elements, except manganese and copper, were significantly affected by germination. Germination of the seed for up to 4 days resulted in significantly higher contents of iron, calcium, magnesium and phosphorus (Table 3).

Total oxalate and phytic acid contents of pigeon pea decreased progressively and attained minimum levels at

Table 3
Mineral composition of *Cajanus cajan* seeds as affected by germination[a]

Germination (day)	Iron (mg/100 g)	Mn (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Cu (mg/100 g)	P (mg/100 g)
0	5.52d[b]	2.94	140c	88.9b	1.06	290d
1	5.55d	3.10	142c	89.0b	1.09	290d
2	6.63c	2.98	164b	92.0b	1.14	298c
3	7.18c	3.16	170a	100ab	1.13	310b
4	8.51b	3.11	172a	104a	1.12	319a
5	9.69a	3.15	170a	108a	1.14	321a
±S.E.M.[c]	0.678	0.037	6.01	3.34	0.013	5.71

[a] Means of four replicate determinations expressed on dry weight basis.
[b] Mean values in a column denoted by different subscripts differ significantly at *P*bkb0.05.
[c] S.E.M., standard error of the mean.

Table 4
Effect of germination on the antinutritional factors in *Cajanus cajan* seeds[a]

Germination (day)	Total oxalate (g/100 g)	Tannins (mg/100 g)	Total phenolics (μg/100 g)	Trypsin inhibitory activity[b]	Phytic acid (mg/100 g)
0	15.4a[c]	2.23b	22.8e	15.4d	811a
1	12.3ab	0.50c	31.7d	16.0d	748b
2	10.3b	0.42c	57.1c	19.3c	575c
3	7.18c	1.89b	85.4b	23.6b	485d
4	3.08d	2.56b	114a	25.3b	306e
5	2.06d	4.28a	115a	39.8a	237f
±S.E.M.[d]	2.142	0.587	16.3	3.68	94.20

[a] Mean of four replicate determinations expressed on dry weight basis.
[b] Expressed as units of enzyme activity inhibited per mg protein.
[c] Mean values in a column denoted by different subscripts differ significantly at $P \leqslant 0.05$.
[d] S.E.M., standard error of the mean.

Table 5
Structural carbohydrates and nutritive and non-nutritive fractions of germinated and ungerminated *Cajanus cajan* seeds[a]

Germination (day)	Nutritive components				Non-nutritive components			Structural carbohydrates		
	Cellular content (%)	Organic cellular content (%)	Soluble ash (%)	Soluble carbohydrate (%)	Acid insoluble ash (%)	Lignin (%)	Non-nutritive matter (%)	Cell wall carbohydrate (%)	Cellulose (%)	Hemicellulose (%)
0	91.7	87.7a[b]	4.05c	63.1a	0.55	1.50a	2.05	8.76	4.23a	4.53d
1	91.4	87.1a	4.27c	63.6a	0.59	1.33a	1.92	8.82	4.13a	4.69d
2	91.2	85.9ab	5.30b	62.1a	0.64	1.28ab	1.92	8.96	3.72b	5.24cd
3	90.6	84.9bc	5.79b	62.3a	0.76	1.07bc	1.83	9.23	3.35bc	5.88bc
4	90.7	84.0bc	6.72a	60.9b	0.84	0.86c	1.70	9.47	2.86cd	6.61ab
5	90.5	83.6c	6.96a	60.4b	0.86	0.80c	1.66	9.80	2.14d	7.66a
±S.E.M.[c]	0.190	0.676	0.495	0.512	0.054	0.113	0.060	0.166	0.327	0.492

[a] Means of four replicate determinations expressed on dry weight basis.
[b] Mean values in a column denoted by different subscripts differ significantly at $P \leqslant 0.05$.
[c] S.E.M., standard error of the mean.

Table 6
Estimated digestible energy (DE) values of germinated and ungerminated *Cajanus cajan* seeds for different laboratory animals[a]

Germination (days)	DE (Kcal/100g DM) obtainable from ungerminated seeds and seeds germinated for the respective number of days by the different laboratory animals:			
	Rabbit	Rat	Swine	Mean
0	320	384	387	364b[c]
1	329	384	391	368ab
2	337	380	387	368ab
3	346	379	390	372a
4	353	376	389	372a
5	359	374	388	374a
			±S.E.M.[b]	1.53

[a] Prediction equations of Fonnesbeck (1976) used for DE estimation are:

Rabbit: DE = 4.67−0.231 NNM (%)−0.0456 CP(%) R^2, 0.971; $S_{y.x}$, 0.101
Rat: DE = 2.54−0.0272 CF (%) + 0.0241 SC (%) R^2, 0.973; $S_{y.x}$, 0.094
Swine: DE = 2.22 + 0.0292 SC (%)−0.129 Lignin (%) R^2, 0.983; $S_{y.x}$, 0.073

[b] S.E.M., Standard error of the mean.
[c] Mean values in a column denoted by different subscripts differ significantly at $P \leqslant 0.05$.

the fifth day of germination. Tannins decreased as the germination progressed for 2 days when they reached the minimum level; thereafter they rose to the highest concentration on the fifth day. Total phenolics and trypsin inhibitory activity in the seeds increased during germination and peaked on the fifth day. The observations made in this study, on the effects of germination on the antinutritional factors, are in agreement with earlier reports. Eskin and Wiebe (1983) reported that germination reduced phytic acid content in germinating seed, due to increased phytase activity. After 48 h of germination, tannin contents of Mung beans (Barroga et al., 1985) and *Dolichos lablab* (Shastry & John, 1991) were reduced by 23–36 and 85%, respectively. Beyond 48 h, tannin content rose progressively in germinating *D. lablab* (Shastry & John, 1991). El-Mandy and El-Sebaiy (1982) and Shastry and John (1991) reported that germination increased trypsin inhibitory activity in legumes. Shastry and John (1991) and Singh and Jambunathan (1981) attributed trypsin inhibition to the phenolic content in the seeds. The increase in the trypsin inhibitory activity in the seed of pigeon pea during germination can be attributed to the increase in the phenolic content of the seed.

In the estimation of the total caloric value of foods, Osborne and Voogt (1978) excluded crude fibre on the assumption that it was indigestible by human digestive enzymes. On the other hand, Fonnesbeck (1976) confirmed partial utilisation of cell wall carbohydrate and declared lignin as the indigestible component of the cell wall carbohydrate. Together with acid-insoluble ash, lignin constituted the non-nutritive matter of the food. Also, Fonnesbeck (1976) established regression equations describing the relationship between nutritive and non-nutritive components of foods and digestible energy values of such foods for different species of animals. Consequently, contents of structural carbohydrates and nutritive and non-nutritive components of pigeon peas that were germinated for 0–5 days were determined (Table 5), and the digestible energy values (Table 6) were estimated from the prediction equations of Fonnesbeck (1976). CC, OCC, SC, cellulose, lignin and NNM were negatively correlated with the duration of germination, whereas SA, AIA, CWC, and hemicellulose were positively correlated with germination (Table 2). Germination resulted in significant reduction in OCC, SC, lignin, NNM and cellulose, while SA increased markedly as the seed germinated. Mean digestible energy value of the pigeon pea was positively correlated with duration of germination, and the value increased significantly, due to germination; optimal increase was achieved in three days of germination.

Based on the results of this study, it may be concluded that germination caused markedly improvements in the caloric value and some valuable nutrients of the seed. However, the increased contents of tannins, total phenolics and trypsin inhibitory activity of the seed during the progressive germination would certainly limit its nutritive quality.

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