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Nutrient Losses Due to Scarification of Pigeonpea (*Cajanus cajan* L.) Cotyledons

U. SINGH, P.V. RAO, R. SEETHA, and R. JAMBUNATHAN

ABSTRACT

Outer layers of cotyledons of pigeonpea cultivar C 11 were successively scarified using a Tangential Abrasive Dehulling Device (TADD). Scarification for 0, 2, 4, 8, and 12 min resulted in the removal of 0, 6.7, 12.7, 25.3, and 36.9%, respectively, of powder fractions. The cotyledons and powder fractions at each level of scarification were analyzed for chemical composition, including minerals and trace elements, protein fractions, amino acid composition, and trypsin inhibitor acitivity (TIA). Protein, soluble sugars and ash of the dhal fraction (scarified cotyledons) decreased with increasing scarification time, while starch content increased. Considerable amounts of calcium (about 20%) and iron (about 30%) were removed by scarification for 4 min, but the process did not adversely affect protein quality in terms of amino acids. Trypsin inhibitors were not removed substantially by scarification.

INTRODUCTION

AMONG FOOD LEGUMES, pigeonpea is an important component of man's diet as a source of protein in several semi-arid and tropical regions of the world. Although India accounts for about 80% of the world's pigeonpea production and consumption, this crop is important in many other countries of Asia and Africa (ICRISAT, 1985)

In India, dehulling of pigeonpea is a primary process that converts whole seed into dhal (decorticated, dry, split cotyledons); various procedures, ranging from commercially operated dhal mills in cities to manually operated stone *chakkis* in the villages, are employed for this purpose (Singh and Jambunathan, 1980). These workers further reported that due to abrasive action of dehulling process, outer layers of the cotyledons are scarified and removed resulting in nearly 12% quantitative yield losses in the form of powder fractions. Attrition-type dehullers and roller mills are particularly suitable for dehulling and splitting legume grains with loose seed coats, whereas, abrasive-type dehullers are suitable for dehulling grains with more tightly adhering seed coats (Kurien, 1984). Using a Tangential Abrasive Dehulling Device (TADD), considerable variability in dehulling quality of cowpea, pigeonpea, and mung bean cultivars was observed by Ehiwe and Reichert (1987).

The distribution of various nutrients in different anatomical parts of legume and cereal seeds has been reported in detail (Singh et al., 1968; O'Dell et al., 1972; Singh and Jambunathan, 1982a). The outer layers of pigeonpea cotyledons are richer sources of protein (Reddy et al., 1979), which is removed during dehulling resulting in considerable losses in protein (Singh and Jambunathan, 1980). Processing methods are known to greatly affect the composition of cereal products, and considerable amounts of nutrients, namely protein, amino acids, minerals and vitamins may be lost if refined cereal products are consumed instead of whole grain products (Pedersen and Eggum, 1983a). However, information on nutrient losses due to dehulling of grain legumes in scanty. As said above, the scarification of outer layers of pigeonpea cotyledons occurs during the dehulling process. The objectives of the present investigation were, to study nutrient (principal chemical constituents, amino acids, minerals and trypsin inhibitors) losses due to scarification of pigeonpea cotyledons and to examine the distribution of such nutrients in dhal (scarified cotyledons) and powder fractions of pigeonpea.

Authors Singh, Rao, Seetha, and Jambunathan are with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, P.O. Box 502 324, Andhra Pradesh, India.

MATERIALS & METHODS

PIGEONPEA CULTIVAR C 11, a popular cultivar in India, was grown in the rainy season 1985/86 at ICRISAT Center, Patancheru 17°N near Hyderabad. This cultivar has an average 100-grain mass of ~10g and a light brown seed coat. After harvest, grains were cleaned and stored for about 3 months at room temperature (25 ± 2°C), a common practice before dehulling of pigeonpea in India. Scarification was carried out using a TADD that was recently developed to simulate large-scale abrasive dehullers (Reichert et al., 1986). Although it is difficult to compare the TADD with a commercially operated dhal mill employed for dehulling pigeonpea, the abrasive action involved in these two dehulling equipments appears to be comparable. The TADD was used to obtain different percentages of dhal and powder fractions, since this would have been difficult to achieve using a commercial dhal mill.

Dehulling

For 0 min scarification time, seeds were not subjected to mechanical abrasive dehulling using the TADD; seed coat was removed manually from the air-dried seeds using forceps. The manually dehulled, unsplit seeds were successively scarified in the TADD for 2, 4, 8, and 12 min to obtain cotyledon and powder fractions. For chemical analysis, scarified cotyledon fractions, hereafter, referred to as dhal fractions in the text, were further ground to a fine powder in a Udy cyclone mill. All chemical analyses were made in duplicate excepting the amino acid composition for which only a single determination was made.

Chemical composition

Protein was determined using a Technicon auto analyzer, as described by Singh and Jambunathan (1982b). Fat, crude fiber and ash were estimated by AOAC (1975) procedures. Soluble sugars and starch were determined according to Thivend et al. (1972). Soluble sugar samples were extracted with 80% hot ethanol. The extracts were evaporated to dryness and the residue was dissolved in distilled water for the estimation of soluble sugars by the phenol-sulfuric acid method (Dubios et al., 1956).

Minerals and trace elements

Samples were digested using a triacid mixture which contained nitric acid, perchloric acid and sulfuric acid in the ratio of 20:4:1. For digestion, defatted samples (0.5g) were weighed and transferred to a block digestor glass tube. After adding 6 mL of triacid mixture, the mixture was digested first at 70°C for 30 min, then at 180°C for 30 min and finally at 220°C for 30 min. After digestion, the mixture was cooled, dissolved in glass distilled water and the volume made to 50 mL. Suitable aliquots were analyzed for calcium, magnesium, zinc, copper, iron and manganese with an atomic absorption spectrophotometer (Varian Tectron Model - 1200) (Piper, 1966).

Amino acid analysis

A sample of 50 mg was refluxed for 24 hr in 50 mL 6 N HCl. After evaporating the HCl from the hydrolyzate, the residue was dissolved in citrate buffer (pH 2.2). The amino acids were analyzed in a Beckman 119 CL amino acid analyzer according to the procedure described by Moore and Stein (1969).

Trypsin inhibitor activity

The trypsin inhibitor activity (TIA) was assayed as per the method described by Kakade et al. (1969). Trypsin inhibitor was extracted by shaking 200 mg of defatted material with 10 mL of 0.1M phosphate buffer (pH 7.6) at room temperature for 1 hr. Protein in the extract was determined according to Lowry et al. (1951).

Protein fractionation

Defatted flour samples were successively extracted with 0.5M sodium chloride in 0.01M phosphate buffer (pH 7.0), 0.1N sodium hydroxide, and 70% ethanol to separate the total proteins into albumin and globulin fractions and glutelin and prolamin fractions, respectively. The flour sample (1 g) was extracted with the first solvent (15 mL) for 1 hr at room temperature; after centrifugation, the residue was reextracted twice with 10 mL solvent each time and supernatants were collected and made up to 50 mL. Similar steps were carried out with the remaining two solvents. Albumin was separaed from the globulin fraction by dialysis. Protein of all the fractions was determined as per the method of Lowry et al. (1951).

Statistical analysis

Two replicates were used for the determination of each constituent. Standard error was determined by "one way analysis of variance" (Snedecor and Cochran, 1967). Standard error indicated in the tables is the pooled error of replications.

RESULTS & DISCUSSION

THE EFFECT OF DURATION of scarification on quantitative yield losses is shown in Table 1. As a result of dehulling, the peripheral layers of the cotyledons were successively removed in the form of powder, resulting in the reduced size of unsplit cotyledons. The size and shape of the unsplit cotyledons of pigeonpea scarified for different intervals are shown in Fig. 1. Although the dhal yield primarily depends on the type of machine, abrasion techniques and other physical conditions that are employed during dehulling, other characteristics such as size, shape and hardness of the grain seem to play an important role in determining dehulling losses (Singh and Jambunathan, 1980).

Protein, soluble sugars, fiber and ash of the powder fractions were considerably higher than those of dhal fractions obtained at different time intervals, whereas the reverse was true for starch (Table 2). These differences were more pronounced with the 2 min scarification time compared to samples of 4 to 12 min sacrification time. This indicated that the outer portions of cotyledons were richer sources of protein, sugar, fiber and ash and poorer sources of the starch, which appeared to be concentrated in the inner layers of cotyledons. By conducting histochemical studies on pigeonpea, Reddy et al. (1979) reported that starch grain size and concentration increased gradually towards the inner layers. They also observed that proteins were concentrated more towards the periphery immediately below the seed coat. Our results also confirmed that scarification led to greater changes in protein content than in other constituents. On the basis of a survey of 36 commercial dhal mills, it was observed that 12.6% dhal was lost during dehulling in the form of the powder fraction (Singh and Jambunathan, 1980). Considering a 4 min scarification time, which was close to a dehulling practice of a commercial dhal mill in terms of powder losses, our present estimates showed that 17.5% of the total pigeonpea dhal protein would be lost due to dehulling as calculated on the basis of relative percentage contributions of the powder protein and dhal protein components to the total protein. This implied that significant protein losses occurred during the dehulling process in commercial dhal mills.

Table 1 – Effect of duration of scarification[a] on dhal (scarified cotyledons) and powder yields of pigeonpea cultivar C 11

Scarification time (min)	100 grain mass (g)	Recovery (%)	
		Dhal	Powder
0	8.4	100.0	–
2	7.9	93.3	6.7
4	7.4	87.3	12.7
8	6.3	74.7	25.3
12	5.0	63.1	36.9
SE	±0.42	±1.30	±0.72

[a] Using the Tangential Abrasive Dehulling Device (TADD) Reichert et al. (1986). Results are averages of two replicates and expressed on a moisture-free basis.

The effects of scarification on minerals and trace elements of dhal and powder fraction are shown in Table 3. Of the various minerals and trace elements, calcium and iron of dhal and powder fractions significantly changed as a result of scarification. Powder fraction appeared to be a rich source of these two constituents. Although magnesium, zinc, copper and maganese were noticeably higher in the powder fraction than in the dhal fraction after 2 min scarification, calcium and iron contents were about three times higher in the powder fraction. This study clearly indicated that these minerals were concentrated in the outer layers of cotyledons and would be lost during dehulling. In cereals, calcium has been reported to be uniformly distributed whereas iron is concentrated in the outer portions of the endosperm (Pedersen and Eggum 1983b). Calcium and iron are important nutrients, but they are deficient in the diets of low-income people particularly infants, preschool children and pregnant and lactating women (Gopalan et al., 1971). For these people, loss of calcium and iron in such processing practices will make these important nutrients unavailable to them.

Amino acids composition, protein fractions and trypsin inhibitors play a very important role in determining the protein quality of grain legumes. The effects of scarification of pigeonpea on protein fractions and trypsin inhibitors are shown in Tables 4 and 5. Results of amino acid analysis of dhal and powder fractions indicated no large differences. The concentration of major amino acids, glutamic acid, aspartic acid, leucine and phenylalanine did not vary between the dhal and powder fractions. Lysine of dhal appeared to be slightly higher than those of the powder fractions, whereas no large differences were observed in the concentration of the sulfur amino acids, methionine and cystine, of dhal and powder fraction. This showed that protein quality in terms of these limiting amino acids might not be adversely affected, although net loss of protein was apparent as a result of dehulling. Pedersen and Eggum (1983b) reported that glutamic acid, proline, aspartic acid, glycine and serine were concentrated in the inner parts of the endosperm of cereals as their concentration increased in the refined flours. But the results of the present study indicated that these amino acids might be uniformly distributed in the cotyledons since no noticable differences in the concentration of these amino acids were observed when the cotyledons were scarified for different intervals. Similar studies are required to know the distribution of amino acids in the cotyledons of other grain legumes, so that such results can be compared with different cereals.

Globulin, glutelin and prolamin fractions of cotyledons scarified for different intervals did not reveal noticeable differences (Table 4). Albumin fraction was higher in the manually decorticated grain (0 min) than in the scarified cotyledons. Trypsin inhibitor activity was slightly reduced as a result of scarification (Table 5). Trypsin inhibitor units per mg sample were higher in the powder fraction than in the dhal, but the trend was reversed when the results were expressed as trypsin

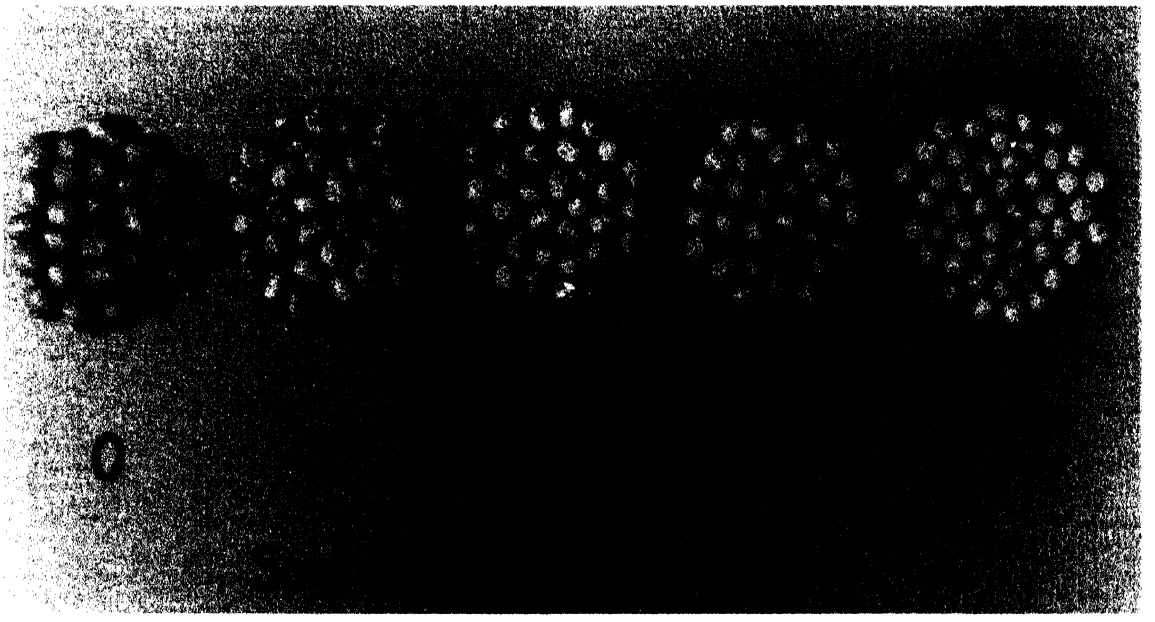

Fig. 1 – Effect of scarification on size and shape of cotyledons.

Table 2 – Effect of scarification on the chemical constituents of dhal (scarified cotyledons) and powder fractions of pigeonpea cultivar C 11[a]

Scarification time (min)	Dhal					Powder				
	Protein	Sugars	Starch	Fiber	Ash	Protein	Sugars	Starch	Fiber	Ash
0	21.4	7.6	62.6	1.1	4.6	–	–	–	–	–
2	20.8	7.2	64.0	1.2	4.4	31.2	10.7	47.0	2.1	5.9
4	20.3	7.0	64.6	1.1	4.2	29.7	10.1	50.9	2.0	5.6
8	19.6	7.2	66.9	1.2	4.2	27.1	9.2	56.2	1.5	5.1
12	19.6	6.9	67.1	1.1	4.1	24.9	8.5	58.7	1.4	4.9
SE	± 0.17	± 0.12	± 0.37	± 0.03	± 0.18	± 0.15	± 0.17	± 0.54	± 0.14	± 0.22

[a] Averages of two replicates and expressed on a moisture-free basis in g/100g

Table 3 – Effects of scarification on mineral and trace elements of dhal (scarified cotyledons) and powder fractions[a]

Scarification time (min)	Dhal						Powder					
	Calcium	Magnesium	Zinc	Copper	Iron	Manganese	Calcium	Magnesium	Zinc	Copper	Iron	Manganese
	(mg/100g)						(mg/100g)					
0	64.9	131.1	3.2	1.6	5.7	1.8						
2	51.7	120.8	3.2	1.5	4.1	1.7	167.8	170.0	5.6	3.4	17.3	3.8
4	51.1	120.6	2.9	1.4	4.0	1.5	116.8	149.8	4.2	2.4	11.9	3.2
8	45.7	122.6	2.8	1.3	3.6	1.5	94.1	122.2	3.9	2.4	9.2	3.2
12	45.1	119.7	2.8	1.2	2.8	1.3	90.4	120.3	3.8	2.2	8.5	2.4
SE	± 2.83	± 3.65	± 0.15	± 0.11	± 0.19	± 0.14	± 2.00	± 3.02	± 0.14	± 0.15	± 1.63	± 0.20

[a] Averages of two replicates, and expressed on a moisture-free basis.

Table 4 – Effect of scarification on seed protein fractions of dhal (scarified cotyledons)[a]

Scarification Time (min)	Albumin	Globulin	Glutelin	Prolamin	Recovery (%)
	(g/100g total protein)				
0	9.5	65.4	18.5	3.5	96.9
2	8.4	67.2	20.3	3.4	99.3
4	8.8	66.5	19.0	2.9	97.2
8	8.2	66.4	18.2	3.2	96.0
12	7.8	66.3	18.2	3.6	95.9
SE	± 0.36	± 1.30	± 0.75	± 0.40	–

[a] Averages of two replicates, and expressed on a moisture-free basis.

Table 5 – Effect of scarification on trypsin inhibitor activity of dhal (scarified cotyledons) and powder fractions[a]

Scarification time (min)	TIU[b]/mg sample		TIU[b]/mg protein	
	Dhal	Powder	Dhal	Powder
0	16.3	–	75.8	–
2	13.9	18.7	66.8	59.9
4	15.0	15.9	73.7	53.0
8	14.5	16.7	74.0	60.4
12	13.8	16.1	74.1	61.5
SE	± 0.54	± 0.32	± 1.48	± 1.52

[a] Averages of two replicates and expressed on a moisture-free basis.
[b] TIU = Trypsin inhibitor unit.

inhibitor units per mg protein. This indicated that the trypsin inhibitors were not removed in proportion to the removal of total protein content during scarification of pigeonpea cotyledons.

CONCLUSION

RESULTS indicated that scarification of pigeonpea cotyledons caused quantitative and qualitative losses. Even though the

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scarified cotyledons contained considerably lower amounts of protein, the process did not adversely affect protein quality of this legume in terms of amino acids. Calcium and iron were removed by scarification. These nutrients are important in the diet of people in the developing countries.

REFERENCES

AOAC. 1975. "Official Methods of Analysis," 12 ed. Association of Official Analytical Chemists, Washington, DC.

Dubios, M., Gilles, K.A., Hamilton, J.K., Robers, P.A., and Smith F. 1956. Colorimetric mehtod for determination of sugars and related substances. Anal. Chem. 28: 350.

Ehiwe, A.O.F. and Reichert, R.D. 1987. Variability in dehulling quality of cowpea, pigeonpea, and mung bean cultivars determined with the Tangential Abrasive Dehulling Device. Cereal Chem. 64: 86.

Gopalan, C., Balasubramanian, S.C., Ramasastri, B.V., and Visweswara Rao, K. 1971. "Diet Atlas of India." National Institute of Nutrition, Hyderabad, India.

ICRISAT 1985. Annual Report. International Crops Research Institute for the Semi-Aird Tropics. 1984, p. 179. Patancheru, A.P. 502 324, India.

Kakade, M.L., Swenson, D.H., and Liener, I.E. 1969. An evaluation of natural vs synthetic substrates for measuring the antitryptic activity of soybean samples. Cereal Chem. 46: 518.

Kurien, P.P. 1984. Dehulling technology of pulses. Res. Ind. 19: 207.

Lowry, O.H. Rosebrough, N., Farr, A.L., and Randall, R.J. 1951. Protein measurement with Folin-Phenol reagent. J. Biol. Chem. 193: 265.

Moore, S. and Stein, W.H. 1969. Chromatographic determination of amino acids by the use of automatic recording equipment. Meth. Enzymol. 8: 819.

O'Dell, B.L., Boland, A.R., and Koirtyohann, S.R. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grain. J. Agric. Food Chem. 20: 718.

Pedersen, B. and Eggum, B.O. 1983a. The influence of milling on the nutritive value of flour from cereal grains. 1. Rye. Qual. Plant. Plant Food Human Nutr. 32: 185.

Pedersen, B. and Eggum. B.O. 1983b. The influence of milling on the nutritive value of flour from cereal grains. 2. Wheat. Qual. Plant. Plant Food Human Nutr. 33: 51.

Piper, C.S. 1966. Mineral analyses by wet digestion with sulphuric acid, nitric acid and perchloric acid. In "Soil and Plant Analysis," p. 272. Hans Publisher, Bombay, India.

Reddy, L.J., Green, J.M., Singh, U., Bisen, S.S., and Jambunathan, R. 1979. Seed protein studies on *Cajanus cajan*. *Atylosia* spp. and some hybrid derivatives. In "International Symposium on Seed Protein Improvement in Cereals and Grain Legumes", vol II, p-105, IAEA, Vienna.

Reichert, R.D., Tyler, R.T., York, A.E., Schwab, D.J., Tatarynovich, J.E., and Mwasaru, M.A. 1986. Description of a production model of the Tangential Abrasive Dehulling Device and its application to breeders samples. Cereal Chem. 62: 201.

Singh, U. And Jambunathan, R. 1980. A survey of the methods of milling and consumer acceptance in pigeonpeas in India. Proceedings of International Workshop on Pigeonpea, Vol 2, p. 419. ICRISAT, Patancheru, A.P. 502 324, India.

Singh, U. and Jambunathan, R. 1982a. Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). Qual. Plant Plant Foods Human. Nutr. 31: 347.

Singh, U. and Jambunathan, R. 1982b. Methods for the estimation of protein in pigeonpea (*Cajanus cajan* [L.] Millsp) and the relationship between whole grain and dhal protein contents. J. Sci. Food Agric. 32: 705.

Singh, S., Singh, H.D., and Sikka, K.G. 1968. Distribution of nutrients in the anatomical parts of common Indian pulses. Cereal Chem. 43: 13.

Snedecor, G.W., and Cochran, W.G. 1967. One-way classifications: Analysis of variance. In "Statistical Methods," 6th ed. p. 260. Oxford & IBH Publishing Co. New Delhi, India.

Thivend, P., Mercier, C., and Guilhot, A. 1972. Determination of starch with glucoamylase. In "Methods in Carbohydrate Chemistry." Whistler R.L. and BeMiller, J.N. (ed.), Vol VI, p. 100. Academic Press, New York.

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