Nutritional and Anti-Nutritional Characteristics of Two Varieties of Red gram (*Cajanus cajan*, L) Seeds

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Abstract- The aim of this study was to investigate the nutritional and anti nutritional factors of the two local varieties of red gram seeds. The results obtained are presented as mean percentage for moisture contents as 8.92, ash, 3.21, dietary fiber, 6.60, protein, 23.23, Fat, 1.45 and total carbohydrate as 53.23 respectively. The study also elicited the mean manganese content to be 1.76, copper 1.80, iron 5.95 and zinc 3.52 mg/100g of dried sample respectively. The mean content of galactosyl oligosaccharides for the two varieties was found to be 1.42%, for raffinose, 1.75% for stachyose and 4.95% for verbascose respectively. Raffinose family sugars in split dhal and immature seeds were found to be 0.85 and 1.00%, for raffinose, 1.54% and 1.11%, for stachyose and 4.20 and 1.38% for verbascose respectively. The mean content of trypsin inhibitor was 199.40 (TIA)/g sample and chymotrypsin inhibitor was 270 (CIU)/g sample.

Index Terms- Red gram, Anti- Nutritional, Oligosaccharides, Trypsin Inhibitor, Chymotrypsin inhibitor.

I. Introduction

Red gram (*Cajanus cajan* L.) or Pigeon pea is among the important grain legumes, which is grown and consumed in the tropics and semi-arid parts of the world (Singh, 1988). Pigeon pea ranks sixth in area and production in comparison to other grain legumes such as beans, peas, and chickpeas (FAOSTAT, 2015). India accounts for about 85% of the world's supply of red gram. Other countries where red gram is an important legume include Kenya, Malaya, Uganda, Thailand, Indonesia and Philippines (Sigh *et al*., 1990). In India, red gram is mostly consumed in the form of dhal (decorticated split cotyledons), after cooking in water, to the desirable degree of softness (Singh, 1988). Among the grain legumes, red gram is a rich source of protein, vitamins, especially the B-complex, and minerals such as calcium and iron (Meiner *et al*., 1976).

Red grams have been shown to contain many antinutritional factors such as trypsin, chymotrypsin inhibitors, polyphenols and galactooligosaccharides (Singh, 1988). These galactooligosaccharides constitute 53% of the total soluble sugars of the grain. However, due to the absence of α-galactosidase activity in the small intestines of human, these sugars cannot be hydrolyzed and absorbed (Olson *et al.*, l981). The undigested raffinose family of sugars (raffinose, stachyose and verbascose) pass into the large intestines, where they are fermented an-aerobically by the microbial flora, which leads to flatus formation and which in turn causes intestinal discomfort, nausea, abdominal rumbling and diarrhea (Liener, 1994). Plant breeding could be one approach for mitigating the flatulence factors, but Ryan & Farmer, (1981) have suggested that eliminating oligosaccharides from the plant could adversely affect the growth and yield of the legumes.

Most of the antinutritional factors, including phytic acid, tannic acid, amylase and proteinase inhibitors, can easily be removed by traditional soaking and cooking methods (Singh 1988). In India, red grams are processed and consumed in a variety of forms. Since little information is available on the nutritional and anti-nutritional content of newly cultivated cultivars of red grams, we endeavor to take up this study.

The proximate and mineral composition of red gram has been studied by a number of researchers. Since the composition is influenced by genetic and environmental factors. It is, therefore, important to evaluate the local grown cultivars in order to assess their nutritional quality. One of the important objectives of this research is to investigate the nutritional and anti nutritional factors of the two varieties of red gram seeds, and oligosaccharide content in split dhal and immature seeds. It is anticipated that the present study will provide useful information for researchers in food and nutrition. By providing the knowledge on the nutritional implication of feeding of raw seeds, split dhal and immature red grams would in turn help ensure the better health condition of people in developing countries.

II. MATERIALS AND METHODS

Red gram cultivars (local 1 and local 2) used in the present study was purchased from a local market and was passed through a 50 mm mesh sieve to remove debris, foreign particles and broken seeds. Standard raffinose, stachyose, and verbascose were purchased from Sigma Chemicals Co. St. Louis MO, USA. All reagents were of analytical grade.

Nutrient analysis

Moisture, ash, crude fibre and fat contents were assayed by the Association of the Official Analytical Chemists (AOAC, 2006) methods 934.01, 942.05, 962.09 and 920.39, respectively. Protein content (N X 6.25) was determined by the AOAC Kjeldahl method (984.13). Carbohydrate was obtained by difference (100 - sum of moisture, protein, fat, crude fibre and ash). Mineral estimation was carried out by dry ashing the sample at 550 ^{0}C according to the AOAC (AOAC, 2006) procedure. Copper, iron, manganese and zinc were determined in a Varian Techtron 100 Atomic absorption spectrophotometer (AAS). The protease inhibitor assay was carried out by casein digestion by following the method of Sumathi & Pattabiraman, (1977).

Determination of oligosaccharide content

The above processed red gram cultivars were milled to flour and passed through a 250 µm sieve. Five gram of each flour was added to Erlenmeyer flasks (250 ml capacity) containing 50 ml of 70% ethanol (v/v), and were placed on an orbital shaker at 130 rpm for 12 h. The contents of the flask were filtered through Whatman No.1 filter paper and the residue was further washed with 25 ml of 70% ethanol. The combined filtrates were evaporated in rotary vacuum evaporator at 40° C. Concentrated sugar syrup was obtained by dissolving the residue left after vacuum evaporation, in 10 ml of distilled water. Ten microlitres of the above syrup were spotted in triplicate on chromatographic plates (19X19 cm) coated with cellulose powder-G (Acme chemicals, Bombay). The plates were kept in a chromatographic chamber containing n-propanol : ethyl acetate : water (6:1:3) as the solvent system (Tanaka *et al.*, 1975). The developed plates were sprayed with 1% α-naphthol in ethyl alcohol containing 10% orthophosphoric acid to identify the sugar spots. For quantitative estimation, an area of (2X3 cm) corresponding to each oligosaccharides was scraped out and soaked in 3 ml of distilled water for 12 h. After 12 h, the mixture was filtered through Whatman No. 1 filter paper and the oligosaccharide content in 1 ml of filtrate was estimated by the method of Tanaka *et al*., (1975).

Estimation of total soluble sugars

Total soluble sugars in the concentrated sugar syrup were estimated by the phenol sulfuric acid method described by Dubois *et al*., (1956). Different volumes (0.2 to1 ml) of glucose as standard and sugar syrup from sample were taken in to series of test tubes and the volume was made up to 1ml with distilled water. One ml of 5% phenol solution and 5 ml of 96% sulphuric acid was added and the content were mixed and placed in water both at 25 to 30^0C for 20 min. The color formed was measured at 490 nm against blank and the concentration of total soluble sugars was calculated using standard curve.

Estimation of reducing sugars

The amount of reducing sugars in the sugar syrup was determined by the method of Nelson, (1944). Different volumes of sugar syrup, 0.2 to 1 ml in to a series of test tubes were taken and the volume was made up to 1ml with distilled water to each tube. One ml of alkaline copper reagent was added and the tubes were placed in boiling water bath for 15 min and cooled under running tap water. To the above 1 ml of arsenomolybdate reagent was added and diluted to 10 ml using 7 ml double distilled water. The optical density of molybdenum blue was measured at 450 nm against blank and the concentration of reducing sugars was calculated using standard curve. The standard curve was constructed using glucose as a standard.

Statistical Analysis

The mean and standard deviation for each fraction in each sample was calculated. The differences in mean values between samples were tested using one-way analysis of variance.

III. RESULTS AND CONCLUSION

Proximate composition of red gram seeds

The nutritional content and anti nutritional factors of the red gram varieties were investigated. The results of the proximate analysis are shown in Table 1. From the table, the carbohydrate in local-1 was found to be 60.23% and local-2 54.23%, respectively. Singh *et al*., (1989) have reported the concentration of various chemical constituents in the whole seeds and dhal sample of pigeon pea and mung bean. Singh *et al*., (1984) have reported the protein and starch together constituted about 75% of the total pigeon pea dhal weight. Megat et al., (2011) have reported the carbohydrate content of kidney bean (27.94%), mung bean (22.34%), soy bean (37.37%) and pea nut (20.06%) respectively.

The protein content in local-1 and local-2 varied considerably and were found to be 25.46% and 21.21% respectively. Singh *et al*., (1989) have reported that the protein content of 43 commonly cultivated varieties of pigeon pea have ranged between 7.9%, 23.3% for whole seeds, and between 21.1% and 28.1% for dhal sample indicating only small variation. The change in the total protein content in the present study also varies with the values reported in the literature; it could be due to (i) type of cultivars, (ii) the methodology employed in the estimation, and (iii) seasonal variations. Megat *et al*., (2011) have reported the protein content in kidney bean as 37.78%, mung bean as 46.09%, soy bean 30.88% and peanut 22.78% respectively.

The fat content of the two varieties of red gram i.e local 1 and 2 are almost similar (1.65% and 1.25%), crude fiber and ash content of the red gram variety local 1 was slightly higher (6.50%, and 3.66%) than that of local 2 (5.50% and 2.46%) respectively. Habibullah *et al*., (2007) have reported that the proximate composition, the moisture, ash, fat and fiber contents of mung bean M1 were found to be 9.4%, 3.9%, 1.9% and 6.8%, respectively, where as in mung bean NM-92 the composition was found to be 8.3%, 3.0%, 2.2% and 7.1% respectively. The crude fiber content of both the varieties is found to be similar when compared with the other commonly cultivated pulses such as chick pea, horse gram and black gram (Premakumare *et al*., 1984). The ash content of local 1 and local 2 is more or less equal to that of *Phaseolus vulgaris* (Tezoto & Sgarbieri, 1990).

Mineral content of red gram seeds

The results of the mineral content (Table 2) analyzed in this study showed that, the red gram varieties local 1 and 2 was higher mean concentration of iron (5.95 mg/100g) than followed by zinc (3.52 mg/100g), copper (1.80 mg/100g) and manganese (1.76 mg/100g) respectively. The mineral contents of both varieties were in good comparison with the results of USDA, (2005) and Mohan & Janardhanan, (1993). The data on mineral analysis revealed that the investigated varieties appear to be a rich source of iron, copper, manganese, and zinc. Both varieties can effectively contribute towards the daily Recommended Dietary Allowances (RDA, 1989) for all groups. It was also observed that generally red gram is used for protein source but it can fulfill the micro nutrients deficiency as well. Sangle, (2015) have reported that minerals constituents of 2 viable mutant varieties of pigeon pea. Mean content of nitrogen ranged from 1.95% to 3.33% and 2.24% to 3.17%, calcium content ranged from 0.25% to 0.37% and 0.26% to 0.51% and phosphorus content of viable mutants ranged from 0.56 % to 0.72 % and 0.58% to 0.80% in varieties of pigeon pea respectively.

Raffinose family oligosaccharide content of red gram seeds

The results of raffinose family sugars were shown in figure 2. From the figure 2 it is evident that the cultivars local 2 had the higher levels of verbascose than that of local 1. The level of stachyose and raffinose were shown a similar concentration in both varieties tested. The sucrose content of the cultivars local 1 and 2 were showed the similar concentration. The values obtained for the levels of sucrose are well within the values reported in the literature. Total soluble sugars in local 1 had the higher levels than that of local 2 varieties and reducing sugar content is more are less similar in both the varieties tested. In Hwa Han & Byung-Kee Baik, (2006) have shown the oligosaccharides, including raffinose, stachyose, ciceritol, and verbascose, are commonly found in legumes and often result in flatulence in humans. Burbano *et al*., (1999) who established that oligosaccharide content was influenced by both variety and environment in *Phaseolus vulgaris*. Stachyose was found to be predominant sugar in lima bean, jack bean and African yam beans while verbascose was predominant oligosaccharide in pigeon pea.

Raffinose family oligosaccharide content of split dhal and immature red gram seeds

The results of raffinose family of oligosaccharides tested in split dhal were shown in Table 3. Dehulling of red gram seeds resulted in a mean decrease of 40.14% for raffinose, 12% for stachyose and 15.15% for verbascose respectively. Dehulling of red gram seeds, also led to a mean decrease of sucrose, total soluble sugars and reducing sugars by 48%, 22.33% and 34.06% respectively. Onyesom et al., (2005) have shown that the dehulling of cowpea reduced the levels of all the soluble sugars such as galactose, glucose, sucrose and raffinose decreased by 3.13%, 31.10%, 0.40% and 13.20% respectively. This observation, suggests a possible localization of all the soluble sugars in the seed coat. Egounlety & Aworh, (2003) have reported that changes in oligosaccharides, trypsin inhibitor, phytic acid and tannins during the pretreatments (soaking and soaking–dehulling–washing–cooking) and fermentation with *Rhizopus oligosporus*. About 50% of raffinose and more than 55–60% of sucrose and stachyose were lost during the pretreatments of the beans. Ruperez, (1998) have shown the oligosaccharides from several types of raw seeds consumed in Spain. The total sugar content ranged from 6.69% to 9.99%, and oligosaccharides represented 25–46% of the total sugar, in the various dry legumes. The main oligosaccharide in raw faba beans was verbascose (3.32%), and stachyose in the remaining legumes (2.21–3.23%).

Results of raffinose family sugars in immature seed were shown in figure 2. From the figure it is evident that the mean concentration of raffinose, stachyose and verbascose in immature seeds was lowest when compared to that of mature and whole red gram seeds. Meiner *et al*., (1976) have reported the green seeds of red gram are used as vegetable in India and generally large seeded cultivars are preferred for the purpose. Jairo *et al*., (1991) have showed that the immature pigeon pea were contain the lowest raffinose family sugar when compared with both mature pigeon pea seeds and with beans or peas, since raffinose family sugars content is increased with seed maturation. They have also reported that very little verbascose was detected in immature seeds.

Trypsin and chymotrypsin inhibitory activity of red gram seeds

The result of trypsin and chymotrypsin which were analyzed showed that the mean levels of chymotrypsin (270 CIU/g sample) were higher than that of trypsin (199.4 TIU/g sample) in both the red gram varieties tested (Table 3). Trugo et al. (1990) from brazil reported higher values of 71–160 TIU mg^{-1} in 10 cultivars of *Phaseolus vulgaris*, samples which is higher when compared to the obtained values. Piergiovanni and Pignone, (2003) found wide variation in the TI content, expressed as TIU mg^{-1} DM (14–39) in 21 local populations of common bean (*P. vulgaris*). They also suggested that drought and thermal stress during vegetative growth may favour increased TI expression. Eva Guillamon *et al*. (2008) have showed that the trypsin inhibition content ranged from negligible in Lupinus spp. to very high in Glycine max. Although there is variation among cultivars, generally the highest TIU mg-1 sample values occured in soybean (43–84) and common bean (21–25). Inhibitor content of different Lathyrus cultivars, ranged from 19–30 TIU mg-1 sample. Onwuka, (2006) have studied the effects of soaking, boiling and combination of soaking and boiling at various treatment levels on the detoxification of trypsin inhibitor, and other anti nutritional factors of pigeon pea. The results showed that the most effective method of detoxifying any of the toxicants was soaking for 12 h and boiling for 80 min. Balogun, (2013) have reported a comparative evaluation of the nutritive value of pigeon pea (*Cajanus cajan*, L.) and cowpea (*Vignaunguiculala,* L.).

Both the varieties local 1 and local 2 contain an appreciable amount of macro and micronutrients which could be included in the daily dietary pattern of every household. This will help minimize the risk of nutrients deficiency in the consumers. Moreover, red grams are palatable, less expensive and abundantly available in the market.

ACKNOWLEDGEMENT

The authors are thankful to the Director, National Institute of Nutrition for the constant support and encouragement.

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Table 1: Proximate composition of red gram seeds (g/100g dry basis). [(a)]

Varieties	Moisture	Protein	Fat	Dietary fiber	Ash	Carbohydrate
Local 1	8.50±1.10	25.46±0.11	1.65±0.04	6.50±0.06	3.66±0.13	54.23±1.50
Local 2	9.35±1.20	21.21±0.52	1.25±0.05	5.50±0.21	2.46±0.03	60.23±2.13
Mean±SD	8.92±0.52	23.23±0.82	1.45±0.06	6.60±0.24	3.21±0.21	53.23±1.81

[(a)]Each Value is average of triplicate determination ±One SD

Table 2: Mineral contents in red gram seeds (mg/100g dry basis) [(a)]

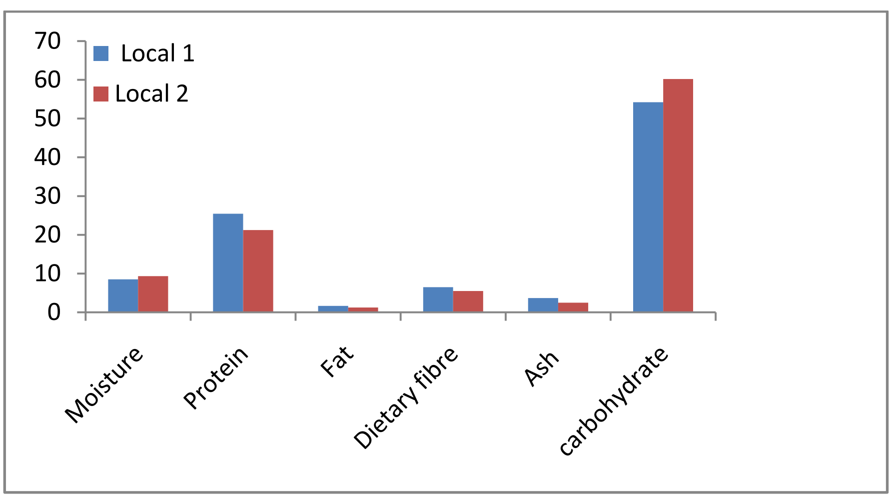
Varieties	Mn	Cu	Fe	Zn
Local 1	1.65±0.20	1.93±0.02	5.80±1.02	3.60±0.04
Local 2	1.88±0.03	1.68±0.05	6.10±0.31	3.44±0.06
Mean±SD	1.76±0.11	1.80±0.03	5.95±0.66	3.52±0.05

[(a)]Each Value is average of triplicate determination ±One SD

Table 3: Trypsin (TIA) and chymotrypsin (CIA) inhibitory activity of red gram seeds.[(a)]

Varieties	Trypsin inhibitory activity (TIU)/g sample	Chymotrypsin inhibitory activity (CIU)/g sample
Local 1	199.68±0.09	281.25±0.25
Local 2	199.12±0.12	199.12±0.12
Mean±SD	199.40±0.10	270.00±0.27

[(a)]Each Value is average of triplicate determination ±One SD



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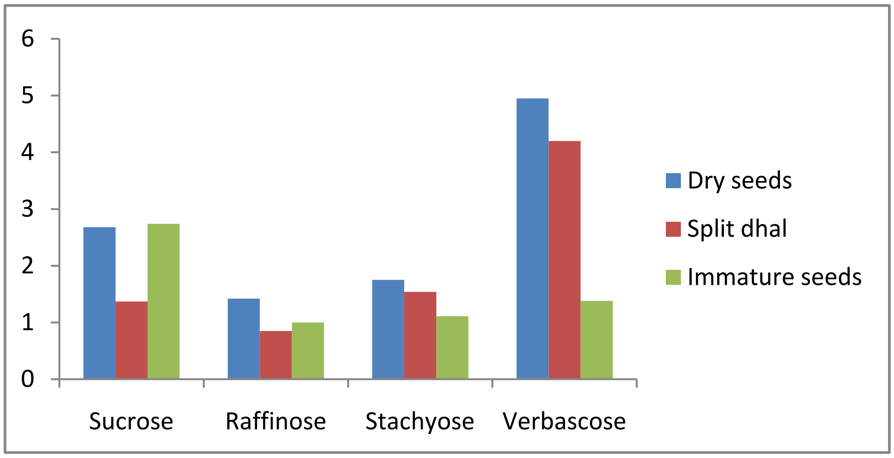

Figure 2: Raffinose family of sugars and sucrose content of dry seeds, split dhal and Immature seeds of red gram (g/100 g dry basis)