Comparing anti-nutritional factors in pigeon pea: seeds vs. Seed coats N.H. GARANIYA*¹, S.M. BAMBHANEEYA², S.G. PATEL³ AND R.P. BAMBHAROLIA⁴

College of Agriculture, Bharuch Campus Navsari Agricultural University Navsari Gujarat India – 392012

Received: September, 2023; Revised accepted: November, 2023

ABSTRACT

The present study investigates the levels of anti-nutritional factors in both the whole seeds and seed coats of various pigeon pea varieties viz. GT-1, GT-101, GT-102, GT-103, GNP-2, VAISHALI, BANAS, AVPP-1, GJP-1, and AGT-2. Anti-nutritional factors such as tannins, phytic acid, oxalic acid, amylase inhibitor, and trypsin inhibitor were analyzed to assess their presence and distribution within the genotypes using standard biochemical methods. The results reveal significant variations in the content of these anti-nutritional factors across different varieties and between whole seeds and seed coats. Notably, the seed coat consistently exhibited higher levels of anti-nutrients compared to the whole seed, emphasizing the need to consider both components when evaluating the nutritional quality of pigeon pea. Among the studied pigeon pea genotypes, AVPP-1 emerged as the most promising variety due to its consistently lower levels of anti-nutritional factors, in both whole seed and its seed coat. These findings underscore the importance of selecting pigeon pea varieties with reduced anti-nutritional factor content to enhance their nutritional value and digestibility. This study highlights the potential for optimizing pigeon pea as a nutritious and healthy food source through targeted breeding and processing techniques, thus contributing to food security and improved nutrition.

Key words: Pigeon pea, Anti-nutrients, Bioavailability, Varieties, Digestibility

INTRODUCTION

Legumes, like pigeon peas, chickpeas, and lentils, are nutritionally rich foods. They typically contain 20-40% protein, with starch as their primary carbohydrate source, making up 50-60% of their composition. Additionally, they contain about 2-3% fat and 0.7-6.2% dietary fiber, along with an array of vitamins and minerals (Wani et al., 2016). They are essential sources of protein, particularly in vegetarian diets (Ghadge et al., 2008), and are widely consumed in many parts of the world (Arinathan et al., 2003; 2009). The remarkable thing about legumes is that they have a low glycemic index and are loaded with bioactive compounds possessina antioxidant properties. which qualifies them as functional foods (Tayade et al., 2019). Furthermore, research by Narina et al. (2014) and Souza et al. (2015) has shown that incorporating legumes into one's diet can have several positive effects on health. These effects include serving as a supplement for individuals with diabetes mellitus, reducing the risk of cardiovascular problems, combating obesity, and promoting bone health. Various countries around the world have adopted legumes as staple foods. South Asia relies on pigeon peas, chickpeas, and lentils, while Latin America

favours kidney beans. North Africa and the Middle East turn to chickpeas, lentils, and faba beans for their nutritional needs (Ahmed and Hasan, 2014). Pigeon pea (Cajanus cajan L.) belongs to the Leguminosae family and is known for its nutritional richness, including crude protein, fiber, vitamins, and antioxidants (Olagunju et al., 2018; Talari & Shakappa, 2018). However, it is worth mentioning that pigeon peas, like other legumes also contains various anti-nutritional factors, such as Phytate, tannins, trypsin inhibitors, cyanogenic alvcosides. hemagglutinins, saponins alkaloids. (Francis et al. 2001; Onwuka et al., 2006: Jukanti et al., 2012: Pele et al., 2016). which can inhibit the bioavailability of nutrients, particularly proteins (Rao, 1998; Aruna, 2016). Anti-nutritional factors (ANFs) are secondary metabolites found in plants that can reduce the utilization of food nutrients (Reddy, 2001). The presence of anti-nutrients in legumes, including pigeon pea, has raised concerns about their impact on human health. For example, phytic acid and oxalic acid can reduce mineral bioavailability, leading to mineral deficiency diseases such as anemia or form complexes with metal ions, such as calcium-oxalate, which can cause renal damage (Deshpande, 1984; 1999: Noonan, Lott 2000). et al.,

Anti-nutrients can be classified into two primary groups: heat-stable and heat-labile (Gemede, 2014). Amylase inhibitors, a heat-labile group, are active in the pH range of 4.5-9.5 (Marshall, 2007). However, these anti-nutrients can be significantly reduced through various processing methods such as fermentation, germination, dehulling, soaking, and boiling. (Duhan et al., 2004; Onwuka et al., 2006). Given the concerns regarding anti-nutritional factors, this study aimed to investigate and compare the levels of various anti-nutrients in the whole seed and seed coat of different pigeon pea varieties.

MATERIAL & METHODS

Seed Samples

experiment The laboratory conducted at the Department of Soil Science Agricultural Chemistry, College Campus. Agriculture. Bharuch Navsari Agricultural University, Gujarat, India in the year 2019 to 2020. Ten pigeon pea genotypes (GT-1, GT-101, GT-102, GT-103, GNP-2, VAISHALI, BANAS, AVPP-1, GJP-1, and AGT-2) were obtained from Mega Seed Pulses and Castor Research Unit, Navsari Agricultural University.

Seed Sample Preparation

The whole seeds and seed coats were separated manually for each pigeon pea varieties. The samples were thoroughly cleaned to remove contaminants, air-dried, and ground to a fine powder using a laboratory grinder. The powdered samples were stored in airtight containers at room temperature until further analysis.

Determination of Anti-nutritional Factors

The levels of various anti-nutritional factors, including tannins, phytate, oxalic acid, amylase inhibitor, and trypsin inhibitor, were determined in both the whole seed and seed coat samples using standard methods. Tannin content was determined according to Sadasivam & Manickam (1992). Phytic acid quantification was done according to Wheeler & Ferrel (1971). Total oxalate was determined according to Day & Underwood (1986) procedure. Amylase inhibitor assay was performed according to the

method of Bernfeld *et al.* (1955). Trypsin inhibitor assay was performed as per the method of Kakade *et al.* (1974).

Statistical Analysis

Experiment was conducted in triplicates. Data were analysed using statistical design of CRD.

RESULTS AND DISCUSSION

The analysis of anti-nutritional factors in pigeon pea seeds and seed coat revealed significant variations within and among different varieties. Table 1 & 2 represents the levels of tannins, phytate, oxalic acid, amylase inhibitor, and trypsin inhibitor in both, the whole seed and seed coat samples.

Tannin

The tannin causes a decrease in the digestibility of protein and carbohydrates due to formation insoluble enzyme-resistant of complexes (Reddy et al., 1998). Deshpande et al. (1986) reported that polyphenols could react with proteins and enzymes and could act as trypsin inhibitors and amylase inhibitors. Result showed that tannin content was in a range of 1.0 mg/g -2.32 mg/g in whole seed. Reena et al., (2018) & lorgyer et al., (2009) supports the same results while Qurat UI Eain Hyder Rizvi et al., (2022) reported the higher tannin content of pigeon pea seed and R. Oloyo, (2002) found very low concentration of tannin. . These results were in agreement with an earlier study of Ene-Obong, (1995) but the level of tannin of whole pigeon pea in the present study seem to be slightly higher as than that observed by Onweluzo, (2009). The variation may be due to varietal differences. Tannin content was 4.40 mg/g – 8.04 mg/g in seed coat.AVPP-1, BANAS, AGT-2 and GJP-1 seeds showed higher tannin content i.e. 2.32mg/g, 2.29 mg/g, 2.23 mg/g and 1.99 mg/g respectively, whereas remaining varieties does not showed significant variation and they had tannin content from 1.0 mg/g -1.25 mg/g. Seed coat of AGT-2 (8.04 mg/g), AVPP-1 (7.97 mg/g), GJP-1 (7.81 mg/g) and BANAS (7.25 mg/g) had highest tannin content while other varieties showed slight variation from 4.40 mg/g - 6.87mg/g.

4.28

Verities	Tannin	Oxalate	Phytate	Amylase Inhibitor	Trypsin Inhibitor
	mg/g	mg/g	mg/g	AIU/g	TIU/g
GT-1	1.10	3.94	5.42	20.09	22.01
GT-101	1.25	3.03	3.39	20.49	19.82
GT-102	1.01	2.99	3.21	22.07	18.71
GT-103	1.23	3.01	2.74	18.12	18.13
GNP-2	1.00	2.02	3.30	23.22	17.43
VAISHALI	1.13	5.01	3.86	31.15	24.07
BANAS	2.29	4.02	2.76	28.50	21.12
AVPP-1	2.32	3.99	2.62	10.82	11.29
GJP-1	1.99	5.01	3.34	27.34	12.49
AGT-2	2.23	5.02	3.08	31.36	12.92
$S.Em \pm (T)$	0.028	0.041	0.143	0.944	0.44
CD @ 5%	0.083	0.122	0.421	2.931	1.298

7.33

Table 1: Anti-nutritional factor content in whole seed of different pigeon pea varieties

1.88

Phytic acid

CV%

Phytate content in whole seed was found from 2.26 mg/g – 5.42 mg/g that was slight lower as compare to report of Reena *et.al.* (2018), R. Oloyo, (2002) and Qurat UI Eain Hyder Rizvi *et. al.*, (2022) and higher than reported by lorgyer et.al. (2009) where as in seed coat it was from 3.37 mg/g – 6.49 mg/g. Egli *et al.*, (2002) and Mulimani *et. al.*, (2003) reported higher values of phytate in millet and pigeon pea. However, the difference may be expected since according to Reddy *et. al.*, (1982), phytate level varies in

3.15

legumes and cereals with variety, cultivar type and soil type among other factors. Highest phytate content was observed in a seed of GT-1 (5.42 mg/g) and rest of the varieties were have almost similar values i.e 2.62 mg/g-3.86 mg/g. Lowest phytate concentration was in AVPP-1 variety. In case of seed coat, GT-1(6.49 mg/g) and VAISHALI (5.15 mg/g) showed highest phytate content and in other varieties, little variation was observed (3.52 mg/g – 4.46 mg/g). However, this result showed that phytate content was higher in all seed coats as compared to whole seeds.

7.38

Table 2: Anti-nutritional factor content in seed coat of different pigeon pea varieties

Verities	Tannin	Oxalate	Phytate	Amylase Inhibitor	Trypsin Inhibitor
	mg/g	mg/g	mg/g	AIU/g	TIU/g
GT-101	5.10	5.05	4.46	27.90	31.58
GT-102	4.99	4.15	4.06	37.62	31.59
GT-103	6.87	5.12	4.14	33.85	27.27
GNP-2	4.40	5.05	4.55	42.63	28.45
VAISHALI	5.62	7.33	5.15	39.32	32.26
BANAS	7.25	7.00	3.52	30.86	28.44
AVPP-1	7.97	6.09	3.37	25.80	25.37
GJP-1	7.81	8.35	3.95	38.45	20.98
AGT-2	8.04	6.12	3.69	37.56	22.97
$S.Em \pm (T)$	0.119	0.226	0.079	0.712	0.404
CD @ 5%	0.352	0.666	0.232	2.099	1.193
CV%	3.22	6.48	3.14	3.6	2.47

Oxalic acid

The results showed oxalic acid in whole seed in a range of 2.02mg/g - 5.02mg/g and in the seed coat, it was from 4.15mg/g - 8.35mg/g. The result indicates that the oxalate content in seed coat is significantly higher that whole seed.

This result of whole seed was accord to the report of lorgyer *et al.*, (2009) wile R. Oloyo, (2002) reported too high concentration of oxalate in pigeon pea. The higher oxalated content in whole seed of AGT-2 (5.02mg/g), GJP-1 (5.01mg/g) & VAISHALI (5.01mg/g) and the lowest was in GNP-2 (2.02mg/g). Whereas seed

coat of GJP-1 (8.35mg/g), VAISHALI (7.33 mg/g) and BANAS (7.00mg/g) had highest value and lowest oxalate was observed in GT-102 (4.15 mg/g).

Amylase inhibitor

From the result it is observed that amylase inhibitor (AI) concentration in whole seed was 10.82AIU/g -31.36AIU/g and in seed coat 25.80AIU/g - 42.67AIU/g. Whole seed of AGT-2 & VAISHALI had highest AI activity i.e. 31.36AIU/g, and 1.15AIU/g, respectively. In other varieties, it was from 18.12AIU/g -28.50AIU/g and the lowest value was observed in AVPP-1 (10.82AIU/g). Highest AI activity was observed in seed coat of GNP-2 (42.63AIU/g). However, Al activity was at par with seed coat of VAISHALI, GJP-1, GT-102 and AGT-2. The lowest seed coat value of Al activity was observed in AVPP-1(27.41AIU/g). In AI activity also, seed coat have significantly higher activity as compared to whole seed.

Trypsin inhibitor

The trypsin inhibitor activity in whole seed was observed from 11.29 TIU/g - 24.07 TIU/g. These results were in the range of trypsin inhibitor activity reported by lorgyer et.al., (2009) & R. Oloyo (2002). Highest value was in VAISHALI and the lowest was in AVPP-I. AGT-2 and GJP-1 were having almost similar value to AVPP-1, i.e. 12.49 TIU/g and 12.92 TIU/g respectively. Activity of trypsin inhibitor in seed coat was from 20.98TIU/g - 34.29TIU/g. The seed coat of GT-1, VAISHALI, GT-102 and GT-101 showed higher trypsin inhibitor activity i.e. 34.29TIU/g, 32.26TIU/a. 31.59TIU/a 31.58TIU/g. Lowest values was observed in GJP-1 seed coat. So, like other parameters, inhibitor activity was also found significantly higher in seed coat as compared to whole seed.

CONCLUSION

This study aimed to determine and compare the content of anti-nutritional factors in the whole seed and seed coat of ten different

varieties of pigeon pea. The results showed that the seed coat contained higher levels of antinutrients compared to the whole seed.

Overall, the study revealed significant variations in the content of anti-nutritional factors among different pigeon pea varieties and between the whole seed and seed coat. These findings highlight the importance of considering the seed coat when assessing the nutritional value and bioavailability of pigeon pea. The study highlights the significance of selecting varieties with lower levels of these undesirable compounds. Among the studied pigeon pea genotypes, one particular variety stands out for its consistently low levels of anti-nutritional factors, making it an appealing choice for various applications. AVPP-1 emerges as the best variety in this context, as it consistently demonstrated lower levels of tannins, phytic acid, oxalic acid, amylase inhibitor, and trypsin inhibitor in its whole seeds and phytic acid, amylase inhibitor, and trypsin inhibitor in seed coats. This remarkable characteristic suggests that AVPP-1 possesses a superior nutritional profile, with improved protein and carbohydrate diaestibility.

The selection of AVPP-1 as the preferred variety is not only beneficial for enhancing the nutritional quality of pigeon pea seeds but also holds promise for broader applications in human diets and livestock feed formulations. Further research and development efforts should focus on exploring the full nutritional potential and utilization of AVPP-1, as it represents a promising choice for addressing anti-nutritional factors and advancing the nutritional value of pigeon pea crops.

conclusion. this study provides valuable insights into the anti-nutritional factors present in pigeon pea varieties and emphasizes the need for further research in this area. Understanding the composition of anti-nutritional factors is crucial for developing strategies to minimize their negative impact on the nutritional value and bioavailability of pigeon pea. By identifying varieties with lower anti-nutritional factor content and exploring methods to reduce their levels, pigeon pea can be optimized as a nutritious and healthy pulse for human consumption.

REFERENCES

- Ahmed, S. and Hasan, M.M. (2014) Legumes: An overview. *Journal of Pharmacy and Pharmaceutical Sciences*, **2**(1), 34-38.
- Aruna, T., & Devindra S. (2016) Nutritional and anti-nutritional characteristics of two varieties of red gram (Cajanus cajan, L) Seeds. *International Journal of Scientific and Research Publications*, **6**(9): 89–95.
- Bernfeld, P. (1955) Amylase α and β. *Methods in Enzymology*, **1**, 149-158.
- Day, R.A., & Underwood, A.L. (1986) *Quantitative Analysis*. 5th Edition, Prentice Hall Publication, Upper Saddle River, 701.
- Deshpande, S.S., Cheryan, M., & Salunkhe, D. K. (1986) Tannin analysis of food products. *Critical Reviews in Food Science and Nutrition*, **24**: 401-449.
- Desphande, S., & Cheryan, M. (1984) Effect of phytic acid, divalent cations, and their interactions on alpha-amylase activity. *Journal of Food Science*, **49**: 516-519.
- Duhan, A., Khetarpaul, N. and Bishnoi, S. (2004) HCl-extractability of zinc and copper as affected by soaking, dehulling, cooking and germination of high yielding pigeon pea cultivars. *Journal of Food Composition and Analysis*, **17**: 597- 604.
- Egli, I., Davidsson, L., Juillerat, M.A., Barclay, D., & Hurrell, R. F. (2002) The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. *Journal of Food Science*, **67**(9):3484-3488.
- Ene-Obong, H.N. (1995) Content of antinutrients and in vitro protein digestibility of the African yambean, pigeon, and cowpea. *Plant Food and Human Nutrition*, **48**: 225-233.
- Francis, G., Makkar, H.P.S. and Becker, K. (2001) Anti-nutritional factors present in plant derived alternate fish feed ingredients and their effect in fish. *Aguaculture* **199**: 197- 227.
- Gemede, H. F., & Ratta, N. (2014) Antinutritional factors in plant foods: Potential health benefits and adverse effects. *Global Advanced Research Journal of Food Science and Technology,* **3**(4): 103-117.
- lorgyer, M.I., Adeka, I.A., Ikondo, N.D., & Okoh,

- J.J. (2009) The Impact of Boiling Periods on The Proximate Composition and Level of Some Anti-Nutritional Factors In Pigeon Pea (*Cajanus Cajan*) Seeds. *Production Agriculture & Technology*, **5**(1): 92-102.
- Jukanti, A. K., Gaur, P. M., Gowda, C.L.L., & Chibbar, R. N. (2012) Nutritional quality and health benefits of chickpea (Cicer arietinum L.): A review. *British Journal of Nutrition*, **108**(S1): S11-S26.
- Kakade, M. L., Rackis, J. J., McGhee, J. E., & Puski, G. (1974) Determination of Trypsin Inhibitor Activity of Soy Products: A Collaborative Analysis of an Improved Procedure. *Cereal Chemistry*, **51**: 376–381.
- Lott, J., Ockenden, I., Raboy, V., & Batten, G. (2000) Phytic acid and phosphorus in crop seeds and fruits: A global estimate. Seed Science Research, 10(1): 11-33.
- Marshall, J. J., & Lauda, C. M. (2007)
 Purification and properties of Phaseolus vulgaris. *Journal of Bioclogical Chemistry*, **250**: 8030-8037.
- Mulimani, V.H., Kadi, N.S., & Thippeswamy, S. (2003) Effect of processing on phytic acid content in different red gram (Cajanus cajan L.) varieties. *Journal of Food Science and Technology*, **40**:371-73.
- Narina, S.S., Bhardwaj, H.L., Hamama, A.A., Burke, J.J., Pathak, S.C. and Xu, Y. (2014) Seed Protein and starch qualities of drought tolerant pigeon pea and native tepary beans. *Journal of Agricultural Science*, **6**(11): 247-259.
- Noonan, S.C., & Savage, G.P. (1999) Oxalic acid and its effects on humans. *Asia Pacific Journal of Clinical Nutrition*, **8**: 64-74.
- Olagunju, A.I., Omoba, O.S., Enujiugha, V.N., Alashi, A.M., & Aluko, R.E. (2018) Pigeon pea enzymatic protein hydrolysates and ultrafiltration peptide fractions as potential sources of antioxidant peptides: An in vitro study. *LWT Food Science and Technology*, **6**(4): 566–574.
- Onweluzo, J.C., & Nwabugwu, C.C. (2009) Fermentation of millet (*Pennisetum americanum*) and pigeon pea (*Cajanus cajan*) seeds for flour production: Effects

- on composition and selected functional properties. *The Journal of Nutrition*, **8**: 737-744.
- Onwuka, G.I. (2006) Soaking, boiling and antinutritional factors in Pigeon peas (*Cajanus cajan*) and cowpeas (*Vigna unguiculata*). *Journal of Food Processing and Preservation*, **30**(5):616 630.
- Pele G.I., Oladiti E.O., Bamidele P.O. and Fadipe, E.A. (2016) Influence of Processing Techniques on the nutritional and anti-nutritional properties of pigeon pea (Cajanus Cajan). International Journal of Engineering and Applied Sciences, 3(1): 92-94.
- Qurat UI Eain Hyder Rizvi, Krishan Kumar, Naseer Ahmed, Ajar Nath Yadav, Divya Chauhan, Priyanka Thakur, & Sumaira Jan. (2022) Influence of soaking and germination treatments on the nutritional, anti-nutritional, and bioactive composition of pigeon pea (*Cajanus cajan L.*). Journal of Applied Biology & Biotechnology, 10(3): 127-134.
- Raheem Oloyo. (2002) Processing effects on the chemical composition and nutritional potentials of the pigeon pea, *Cajanus cajan*, L. *Rivista Italiana Delle Sostanze Grasse*, **79**: 273-276.
- Rao, P., & Desothale, Y. G. (1998) Tannin content of pulses varietal difference and effects of germination and cooking. *Journal of the Science and Agriculture*, 33: 1013-1015.
- Reddy, N.R., & Sathe, S.K. (2001) *Food Phytates (1st ed.)*. CRC Press. https://doi.org/10.1201/9781420014419
- Reddy, N.R., Pierson, N.D., Sathe, S.K., & Salunkhe, D.K. (1998) Dry bean tannins a review of nutritional implications.

- Journal of the American Oil Chemist Society, **62**: 541-549.
- Reddy, N.R., Sathe, S.K., & Salunkhe, D.K. (1982) Phytates in legumes and cereals. *Advances in Food Research*, **28**: 1-92.
- Reena Devi, Charul Chaudhary, Veena Jain, AK Saxena, & Shilpa Chawla. (2018) Effect of soaking on anti-nutritional factors in the sun-dried seeds of hybrid pigeon pea to enhance their nutrients bioavailability. *Journal of Phytoccognocy and Phytochemistry, 7(2): 675-680
- Sadasivam, S., and Manickam, A., (1992) Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd., New Delhi.
- Souza, R.G.M., Gomes, J.A.C., Naves, M.M.V. and Mota, J.F. (2015) Nuts and legume seeds for cardiovascular risk reduction: Scientific evidence and mechanism of action. *Nutrition Reviews*, **73**(6), 335-347.
- Talari, A., and Shakappa, D., (2018) Role of pigeon pea (*Cajanus cajan L.*) in human nutrition and health: a review. *Asian Journal of Dairy and Food Research*, **37**(3): 212–220.
- Tayade, R., Kulkarni, K.P., Jo, H., Song, J.T. and Lee, J.D. (2019) Insight into the prospects for the improvement of seed starch in legume A review. *Frontiers in Plant Science*, **10**: 1213.
- Wani, I.A., Sogi, D.S., Hamdani, A M., Gani, A., Bhat, N.A. and Shah, A. (2016) Isolation, composition, and physicochemical properties of starch from legumes: A review. *Starch*, **68**(9-10), 1-12.
- Wheeler, E. L., and Ferrel, R. E. (1971) A Method for Phytic Acid Determination in Wheat and Wheat Fractions. *Cereal Chemistry*, **48**:312 320.