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OPTIMIZATION OF HYDROTHERMAL TREATMENT ON ANTINUTRITIONAL FACTORS IN SELECTED PIGEON PEA VARIETY IN MYANMAR

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Abstract:

The presence of anti-nutritional factors negatively affects the bioavailability of protein, calcium, iron and zinc. This study was conducted with the aim of optimizing the effect of hydrothermal treatment and drying on antinutritional factors and protein content in pigeon pea. The Central Composite Rotatable Design of RSM was applied to optimize the hydrothermal condition for reducing antinutritional factors. The experiment was conducted with 2 independent factors (hydrothermal treatment and drying and drying) at five levels (− α,−1, 0, 1, + α). The optimum conditions for the lowest antinutritional factors in pigeon pea were 11-minute hydrothermal treatment and drying and 2-h drying.

Key Words- Pigeon pea, anti-nutritional factors, hydrothermal treatment and drying, optimization, Response Surface Methodology (RSM), Central Composite Rotatable Design (CCRD)

Introduction

Pigeon pea *Cajanus cajan* is a nutritionally important grain in the tropics (Rampersad et al., 2003). It is rich in protein (19 - 26 %) and minerals (Rampersad et al., 2003). It contains 20-26% protein, 1-2% fat, 53 -65% carbohydrate, and 3.8-8.1% ash (Ajayi et al., 2010; Saxena and Kumar,

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2010). Pigeon pea varieties has protein content in the range of 23 - 26% (Oshodi et al.,1993). But, within pigeon pea cultivars, anti-nutritional factors are mainly found among dark-seeded genotypes (Farris and Singh, 1990) that are generally grown in Asia. The anti-nutritional factors found in pigeon pea include trypsin inhibitors, chymotrypsin inhibitors, amylase inhibitors, hemagglutinins (lectins), tannins (polyphenols), saponins, cyanide, phytic acid, oxalate (Farris and Singh, 1990; Nwosu., 2013). Antinutritional factors are those substances, which either by themselves or through their metabolic products, interfere with nutrient utilization and cause serious health issues, which include reduction in nutrient intake, digestion, absorption and utilization and may produce other adverse effects (Akande & Fabiyi, 2010). The reduction of undesirable and toxic compounds present in high concentrations in seeds **is** essential to improve the nutritional quality and effective utilization of them in different foods (Akande & Fabiyi, 2010).

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes (Myers & et.al., 2016). In recent years, several advances have been made in optimizing for reducing antinutritional factors using response surface methodology (RSM). Optimizing refers to improving the performance of a system, a process, or a product in order to obtain the maximum benefit from it. The significant advantage of RSM is that it allows the evaluation of the independent variables and their interactions on the dependent variables with a reduced number of trials (Alara, Abdurahman, & Olalere, 2018). RSM is often used to optimize the processing parameters and has the advantages of fewer experiments and higher accuracy. Hydrothermal treatment and drying (HTT) was investigated as an alternative method for antinutritional factors reduction in pigeon pea.

2. Objective

The focus of this work was to study the effect of hydrothermal treatment and drying in reducing the antinutritional factors present in pigeon pea varieties and to enhance its utilization in food systems as a rich source of plant protein.

3. Materials and Sample Preparation

The seeds of five pigeon pea varieties, namely; Monywa Shwe Dinga, Yezin-5, Yezin-8, Yezin-9, and Yezin-10, were obtained from the Regional Research Center (Zaloke), Department of Agricultural Research, Monywa Township, Sagaing Region, Myanmar. All of the pigeon pea seeds were dry-cleaned and particles such as stalks, pebbles, and immature and broken seeds were removed. They were then packaged in labelled plastic containers. Five-hundred grams of pigeon pea seeds were ground with Panasonic AC 400 Mixer Grinder and to obtain flour, all are sieved by using a 1-mm-mesh size sieve. The pigeon pea powders were packed in good-quality aluminium foil and then placed in an air-tight bottle at ambient temperature until it was analysed.

Distilled water from PHRI Yezin, Department of Agriculture, Ministry of Agriculture, Livestock and Irrigation was utilized for the whole experiment. In addition, all the reagents used

were analytical grade and chemicals from Qualikems Fine Chem Pvt. Ltd https://qualikems.com/ and mp biomedicals, LLC https://www.mpbio.com/us were applied. The entire experiment was carried out at the Department of Postharvest Technology, ACARE, Yezin Agricultural University from April 2022 to June 2022.

3.2 Statistical Analysis

Response surface methodology (Design Expert Version 12) was used to optimize the processing conditions at five levels, and two variables. These treatments were carried out with thirteen experiments of CCRD design. The treatment combination of hydrothermal treatment and drying and drying.

3.3 Experimental Design

The Central Composite Rotatable Design of response surface methodology was applied to optimize the reduction of antinutritional factors. The experiment was carried out with two independent variables like hydrothermal treatment and drying and drying each at five levels; namely (−α,− 1, 0, 1, +α). The adequacy of each model was determined by evaluating the lack of fit and the coefficient of determination (R^2). The significance of each coefficient was determined by using F-test obtained from the analysis of variance (ANOVA) that was generated. Regression coefficients were then used to generate response surfaces. 3D response surface graph for the predicted value and variable was plotted using the Design Expert 12 software. Thirteen experiments were performed which consisted of two factorial points, two extra points (star points) and five replicates for the center point. The five replicates for the center point were used to estimate the experimental error.

3.4 Determination of Selected Antinutritional Factors

(a) Phytic acid

Phytic acid was determined by the method of Wheeler & Ferrel (1971) with slight modification. A finely ground sample (3 g) was extracted with 50 ml of 0.8 M of HCl for 45 minutes in a shaker. The content was centrifuged at 3000 rpm for 15 minutes and a 10 ml aliquot of the supernatant was transferred to a 40-ml conical centrifuge flask. Ferric chloride solution (4 ml) was added rapidly to the aliquot and heated in a boiling water bath for 45 minutes. During this 45 minute, after 30 minutes, one to two drops of sodium sulphate in 0.8 M HCl was added and heating was continued for another 15 minutes, so it takes total 45 minutes.

The contents were then centrifuged at 3000 rpm for 15 minutes and the supernatant was carefully decanted. The precipitate was washed well by dispersing it with 25 ml of 0.8 M HCl and heated in a boiling water bath for 10 minutes, and centrifuged at 3000 rpm for 15 minutes. The supernatant was again decanted carefully and discarded, and the precipitate was washed with distilled water. The precipitate was dispersed with distilled water and 3 ml of 1.5 N NaOH was added. The contents were brought to about 30 ml with distilled water and heated in a boiling water bath for 30 minutes. The contents were filtered through Whatman No. 4 filter paper. The precipitate was washed with 70 ml of hot water. The precipitate on the filter paper was dissolved with 40 ml of

hot 3.2 N nitric acid in a 100 ml volumetric flask. The filter paper was washed several times with water and all the washings were collected in the same flask. The contents of the flask were cooled down and the volume was made up with distilled water up to 100 ml. The 5 ml aliquot was transferred to a new 100 ml volumetric flask. After that 70 ml of water was added into 20 ml of 1.5 M potassium thiocyanate (KSCN) and the volume made up with distilled water, and the colour was read at 480 nm through spectrophotometer (UV-2600, Shimadzu Corporation, Kyoto, Japan). Phytic acid was calculated using the following equation:

$$\boldsymbol{Phytic\ acid(mg/100g) = \frac{4}{6} x \frac{mgFex10x20x50}{sample\ weightx1000} x100}$$ **(**Wheeler & Ferrel,1971)

(b) Oxalate

Samples were prepared for estimation of oxalate content by spectrophotometric method described by (Naik *et. al.*, 2014).

Preparation of standard oxalic acid solution: (1mg/ml) A standard solution of oxalic acid was prepared by dissolving 100 mg of oxalic acid in distilled water and diluted to 100 ml with distilled water.

Working solution: From the standard oxalic acid solution, 0.1, 0.2, 0.3 and so on up to 1mg/ml concentration solution were prepared by proper dilution. 0.003 M KMnO4 (100 ml Molecular weight of $KMnO_4$ is 158. So, 0.048 g $KMnO_4$ was added to 100 ml water for the preparation of 0.003 M $KMnO_4$. 2 N H_2SO_4 (500 ml) [S.D Fine Chemicals, Mumbai] Concentrated H_2SO_4 was taken with purity of 98% (specific gravity= 1.84 GPL). So, 27.2 ml H_2SO_4 was added to 500 ml distilled water to make 2N H_2SO_4.

Preparation of graded standard solution for reading A series of standard solutions of oxalic acid containing 0.1, 0.2, 0.3 and so on up to 1 mg/ml were mixed with 5ml of 2N H_2SO_4 and 2ml of 0.003 M $KMnO_4$ in separate test tubes and incubated at 37° C for 10 minutes.

Preparation of blank - Blank sample was prepared by adding 5ml of 2N H_2SO_4 and 2ml of 0.003 M $KMnO_4$ simultaneously with solution mixture and incubated at 37° C for 10 minutes. 0.25 N HCl (100 ml). Next (2.1) ml concentrated HCl was added to 100 ml distilled water to make 0.25 N HCl.

Preparation of sample extract- 0.5g of plant material was weighed with digital balance and transferred to 30 ml of 0.25 N HCl and was boiled in a water bath for 15 minutes. The total extract volume was then made to 50 ml by adding already prepared 0.25 N HCl.

Preparations of sample extract mixture for reading - 1 ml of sample extract was added with 5ml of 2 N H_2SO_4 and 2ml of 0.003 M KMnO4 at the same time of preparation of other solution mixture for reading and incubated at 37° C for 10 minutes.

(c) Saponin

Saponin content was determined using the method reported by Ejikeme *et. al.*, 2014 and Obadoni & Ochuko, 2002. Five gram of ground pigeon pea sample was put into a 250 cm3 conical flask and exactly 100 cm3 of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The mixture was filtered and the residue re-extracted with another 100cm3 of 20% aqueous ethanol, heated for 4 hours at a constant temperature of 55o C with constant stirring. The combined extract was evaporated to 40 cm3 in water bath at a temperature of 90° C. The concentrate was transferred into a 250cm3 separator funnel and 20cm3 of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated twice. 60cm3 of n-butanol was added and the butanol extract was washed twice with 10cm3 of 5 % sodium chloride. The sodium chloride layer was discarded and the remaining solution heated in a water bath for 30 minutes. The solution was then transferred into a crucible and was dried in an oven to a constant weight.

4. Results and Discussion

Table 1 Experimental range and levels of the independent variable for Central Composite Rotatable Design (CCRD) of response surface methodology (RSM) for hydrothermal treatment and drying

Independent variables				**Level of range**				
Factors	Unit	Notation	Model	-α	1	0	1	+α
				-1.41421	Low	Medium	high	1.41421
Hydrothermal treatment and drying	in	A	X_1	2.92893	5	10	15	17.0711
Drying	h	B	X_2	1.58579	2	3	4	4.41421

Table 2 Coded and actual values of hydrothermal treatment and drying parameters in the experimental Central Composite Rotational Design

Coded Value			Actual Value	
Test runs	X_1 Hydrothermal treatment and drying **(min)**	X_2 **Drying (h)**	X_1 Hydrothermal treatment and drying **(min)**	X_2 **Drying (h)**
1	0	0	10	3
2	+1	+1	15	4
3	+1	-1	15	2
4	0	0	10	3
5	0	0	10	3
6	0	0	10	3
7	-1	-1	5	2
8	0	+α	10	4.5
9	-α	0	3	3
10	+α	0	17	3
11	-1	+1	5	4
12	0	+1	10	1.5
13	0	0	10	3

Table 3 Experimental design and responses obtained from CCRD

Experimental Runs	Hydrothermal treatment and drying (min)	Drying (h)	Protein (%)	Phytic acid (mg/ 100 g)	Oxalate (mg/ 100 g)	Saponins (mg/ 100 g)
1	10	3	23.56	287.38	183.63	1376.60
2	15	4	23.20	343.19	220.99	1504.05
3	15	2	23.75	286.93	186.66	1382.31
4	10	3	23.50	287.38	185.77	1328.32
5	10	3	23.50	287.38	185.77	1316.60
6	10	3	23.50	272.57	185.77	1316.65
7	5	2	23.46	294.59	202.32	1394.65
8	10	4.5	23.34	344.53	201.92	1604.71
9	3	3	23.55	282.46	196.34	1380.72
10	17	3	23.46	310.82	209.08	1384.49
11	5	4	23.61	296.02	182.33	1497.33
12	10	1.5	23.65	293.54	186.69	1413.14
13	10	3	23.50	272.57	185.77	1316.65

Table 4 Regression coefficient for fitted quadratic model of product responses

Independent variables	Dependent variables			
	Protein (%)	Phytic acid (mg/100 g)	Oxalate (mg/100 g)	Saponin (mg/100 g)
Intercept of model Linear effect	23.512	284.419	185.342	1330.96
Hydrothermal treatment and drying (A)	-0.03 **	9.95 **	5.13 **	-0.03 ns
Drying (B)	-0.10 **	16.22 **	4.48 **	61.91 **
Interaction effect				
AB	-0.175 **	13.71 **	13.58 **	4.77ns
Quadratic effect				
A^2	-0.00 ns	5.44 **	8.57 **	25.53 **
B^2	-0.00 ns	16.64 **	4.37 **	88.69 **

*** significant at probability level < 0.0001; ** significant at probability level p < 0.01;

* significant at probability level p < 0.05; ns non-significant

Table 5 Analysis of Variance for the fit of experimental data to quadratic model

Factors	Protein (%)	Phytic acid (mg/100 g)	Oxalate (mg/100 g)	Saponins (mg/100 g)
Model F- value	97.96***	36.72***	174.29***	40.60***
Lack of Fit (P value)	0.95ns	0.82ns	0.12ns	0.93ns
R^2	0.99	0.96	0.99	0.97
CV %	0.089	1.86	0.72	1.48

*** significant at probability level < 0.0001; ** significant at probability level p < 0.01; * significant at probability level p < 0.05; ns non-significant; R^2 = coefficient of determination; CV % = coefficient of variation

Table 6 Desired goals for responses, optimum process parameters and predicted response values

Name	Desired Goal	Predicted value
Process parameters		
Hydrothermal treatment and drying (min)	in range (5-15)	11
Drying (h)	in range (2-4)	2
Response variables		
Protein (%)	Maximum 23.75	23.64
Phytic acid (mg/100 g)	Minimum 272.54	283.14
Oxalate (mg/100 g)	Minimum 182.33	183.49
Saponins (mg/100 g)	Minimum 1316.60	1348.69
Desirability	92%	

Table 7 Confirmation report for optimized level and the respective responses of using point prediction

Responses	Predicted value	Actual value	Residual error
Protein (%)	23.64	23.27	-0.37
Phytic acid (mg/100 g)	283.14	290.00	6.86
Oxalate (mg/100 g)	183.49	187.27	3.78
Saponins (mg/100 g)	1348.69	1356.72	8.03

Table 8 Reduction percentage of antinutritional factors after hydrothermally treated in pigeon pea variety of Yezin - 5

Antinutritional factors								
Phytic acid (mg/ 100 g)			**Oxalate (mg/ 100 g)**			**Saponin (mg/ 100 g)**		
Initial value	After hydrothermal treatment and drying	% Reduction	Initial value	After hydrothermal treatment and drying	% Reduction	Initial value	After hydrothermal treatment and drying	% Reduction
561.98	272.57	37.18~58.43	341.83	192.40	32.77~62.43	2074	1316.60	21.25 ~ 40.55

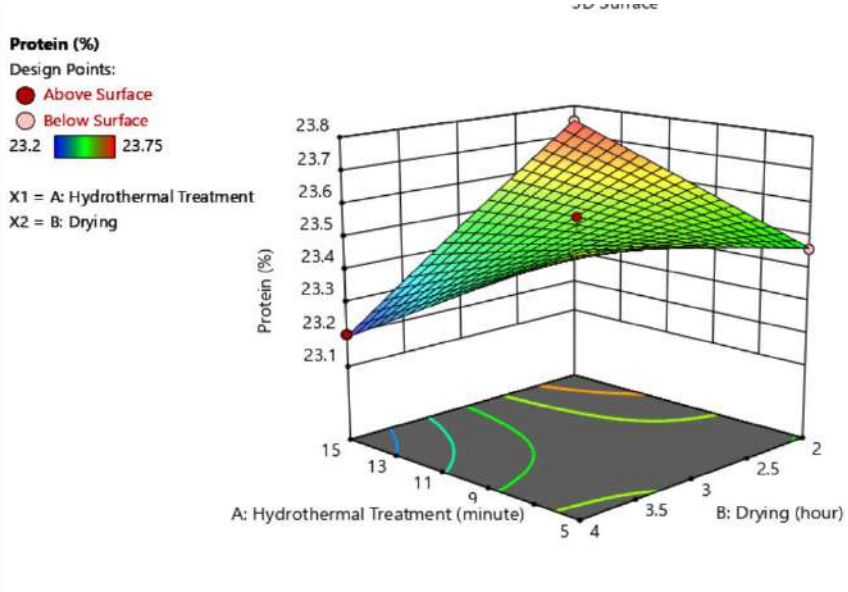

Figure 1 Three-dimensional response surface plots for h hydrothermal treatment and drying and drying as a function of Protein (%)

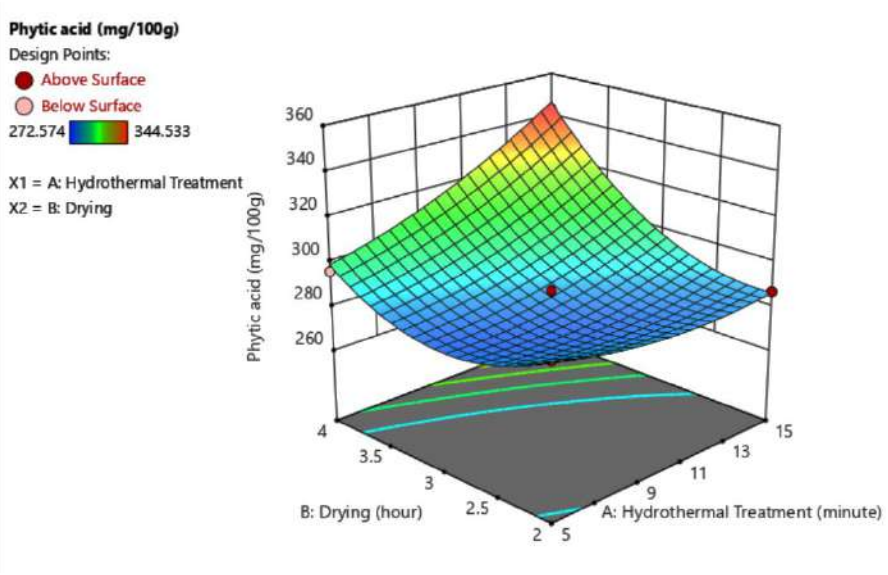

Figure 2 Three-dimensional response surface plots for h hydrothermal treatment and drying and drying as a function of Phytic Acid (mg/100g)

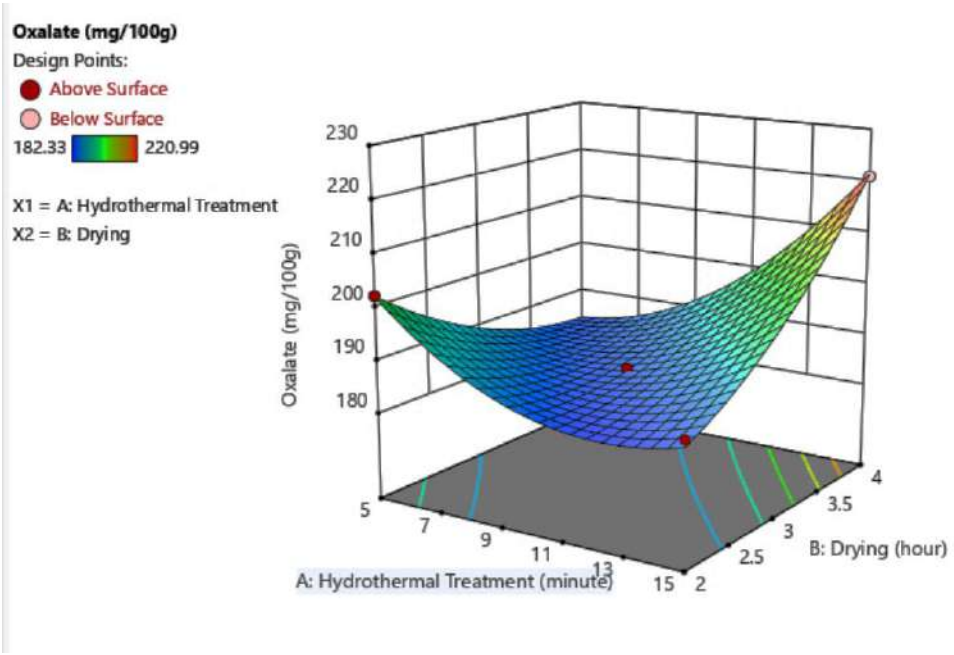

Figure 3 Three-dimensional response surface plots for h hydrothermal treatment and drying and drying as a function of Oxalate (mg/ 100 g)

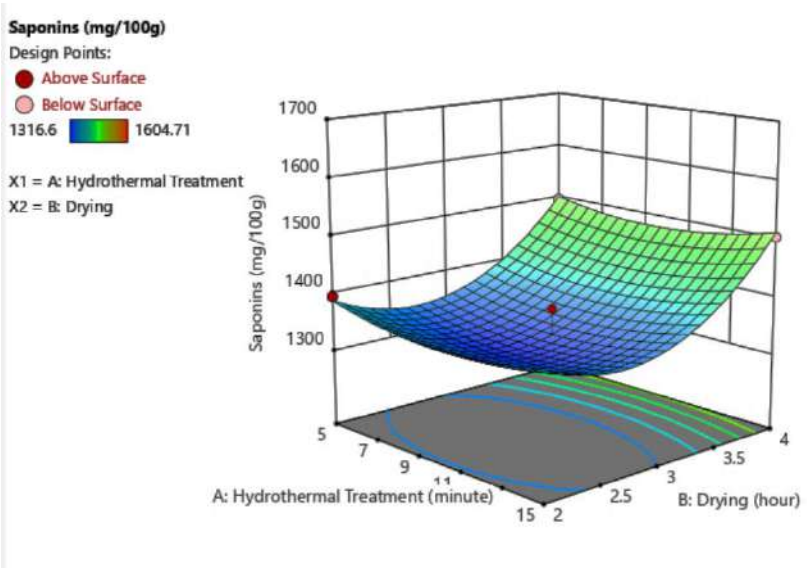

Figure 4. Three-dimensional response surface plots for h hydrothermal treatment and drying and drying as a function of Saponins (mg/ 100 g)

5. Conclusion

The findings of this study showed that pigeon pea is a great source of plant protein; however, it is unexplored because of the presence of anti-nutritional factors. Typically, a better understanding of the effect of different processing techniques on the nutritive value and antinutritional factors may lead to the use of pigeon pea on a wider scale in the food industries. Compared to untreated pigeon pea, hydrothermal treatment and drying significantly decreased antinutritional factors.

Considering the effect of hydrothermal treatment and drying, a remarkable reduction in antinutritional factors was achieved. In summary, although a complete removal of antinutritional factors was not reported, hydrothermal treatment and drying can help reduce anti-nutritional factors to a particular extent. The optimized conditions were 11-min hydrothermal treatment and drying and 2-h drying. At these conditions, there was reduction of phytic acids, oxalate and saponins by 58.43%, 62.43% and 40.55% respectively.

Hence, hydrothermal treatment and drying can be considered as a useful technique for reducing the antinutritional factors as well as improving the nutritional quality of pigeon pea. Based on the findings of this study, the best-optimized condition of hydrothermal treatment and drying (11-min hydrothermal treatment and 2-h drying) for pigeon pea should be used as a pre-treatment method to reduce antinutritional factors without reducing protein content.

6. Conflict of interest The author has no conflict of interest.

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8. References

1. Ajayi, F. T., Akande, S. R., Odejide, J. O., & Idowu, B. (2010). Nutritive evaluation of some tropical under-utilized grain legume seeds for ruminant's nutrition. *Journal of American Science*, *6*(7), 1-7.
2. Akande, K. E., & Fabiyi, E. F. (2010). Effect of processing methods on some antinutritional factors in legume seeds for poultry feeding. http://repository.futminna.edu.ng:8080/xmlui/handle/123456789/15821
3. Alara, O. R., Abdurahman, N. H., & Olalere, O. A. (2018). Optimization of microwave-assisted extraction of flavonoids and antioxidants from Vernonia amygdalina leaf using response surface methodology. *Food and bioproducts processing*, *107*, 36-48. https://www.sciencedirect.com/science/article/pii/S0960308517301323
4. Champ, M. M. J. (2002). Non-nutrient bioactive substances of pulses. *British Journal of Nutrition*, *88*(S3), 307-319.

5. Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *The American journal of clinical nutrition*, *72*(6), 1424-1435.
6. Ejikeme, C., Ezeonu, C. S., & Eboatu, A. N. (2014). Determination of Physical and Phytochemical Constituents of some Tropical Timbers Indigenous to nigerdelta area of nigeria. *European Scientific Journal*, *10*(18), 247-270. http://eprints.gouni.edu.ng/1312
7. Faris, D. G., & Singh, U. (1990). Pigeonpea: nutrition and products. *Pigeonpea: nutrition and products.*, 401-433. https://www.cabdirect.org/cabdirect/abstract/19911437454
8. https://books.google.com/books?hl=en&lr=&id=ps8YYavx57EC&oi=fnd&pg=PA1&dq=Tiwari,+B.+K.,+%26+Singh,+N.+(2012).+Pulse+chemistry+and+technology.+Cambridge:+RSC+Publishing.&ots=OR6S5vSfWZ&sig=r8iF-5a1A6-QfQsPJ3GczFN8hF8
9. https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/nonnutrient-bioactive-substances-of-pulses/188E86250C8931EE94D2AB498E1D3B01
10. Lutaladio, N., & Castaldi, L. (2009). Potato: The hidden treasure. *Journal of food composition and analysis*, *22*(6), 491-493. https://www.sciencedirect.com/science/article/pii/S088915750900132X
11. Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2016). *Response surface methodology: process and product optimization using designed experiments*. John Wiley & Sons.
12. Naik, V. V., Patil, N. S., Aparadh, V. T., & Karadge, B. A. (2014). Methodology in determination of oxalic acid in plant tissue: a comparative approach. *Journal of Global Trends in Pharmaceutical Sciences*, *5*(2), 1662-1672. https://www.cabdirect.org/cabdirect/abstract/20143229078
13. Nwosu, J. N., Ojukwu, M., Ogueke, C. C., Ahaotu, I., & Owuamanam, C. I. (2013). The Antinutritional Properties and Ease of Dehulling on theProximate Composition of Pigeon pea (Cajanus cajan) as Affected by Malting. *International Journal of Life Sciences Vol. 2*, *2*(2013), 60-67. http://www.crdeepjournal.org/wp-content/uploads/2013/05/Vol-2-2-1-IJLS.pdf
14. Oshodi, A. A., Olaofe, O., & Hall, G. M. (1993). Amino acid, fatty acid and mineral composition of pigeon pea (Cajanus cajan). *International Journal of Food Sciences and Nutrition*, *43*(4), 187-191. https://www.tandfonline.com/doi/abs/10.3109/09637489309027541
15. Rampersad, R., Badrie, N., & Comissiong, E. (2003). Physico-chemical and sensory characteristics of flavored snacks from extruded cassava/pigeonpea flour. *Journal of food science*, *68*(1), 363-367. https://ift.onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2621.2003.tb14166.x
16. Saxena, K. B., Kumar, R. V., & Sultana, R. (2010). Quality nutrition through pigeonpea-a review. *Health*, *2*(11), 1335-1344. http://eprints.icrisat.ac.in/809/
17. Shi, L., Mu, K., Arntfield, S. D., & Nickerson, M. T. (2017). Changes in levels of enzyme inhibitors during soaking and cooking for pulses available in Canada. *Journal of food science and technology*, *54*, 1014-1022. https://link.springer.com/article/10.1007/s13197-017-2519-6
18. Singh, B., Singh, J. P., Singh, N., & Kaur, A. (2017). Saponins in pulses and their health promoting activities: A review. *Food Chemistry*, *233*, 540-549. https://www.sciencedirect.com/science/article/pii/S0308814617307380
19. Tiwari, B. K., & Singh, N. (2012). *Pulse chemistry and technology*. Royal Society of Chemistry.
20. Wheeler, E. L., & Ferrel, R. E. (1971). A method for phytic acid determination in wheat and wheat fractions. *Cereal chemistry*, *48*(3), 312-320. https://www.cabdirect.org/cabdirect/abstract/19721492732

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