Magna Scientia Advanced Biology and Pharmacy
eISSN: 2582-8363
Cross Ref DOI: 10.30574/msabp
Journal homepage: https://magnascientiapub.com/journals/msabp/

Magna Scientia Advanced Biology and Pharmacy (MSABP)
Magna Scientia
Open access publication

(Research Article)

Check for updates

Comparative evaluation of the effect of boiling and autoclaving of legume grains on tannin concentration

Donald Mudenda Chisowa *

Department of Animal Science, School of Agriculture, Southern University, P. O Box 60293, Livingstone, Zambia.

Magna Scientia Advanced Biology and Pharmacy, 2022, 07(01), 009–017

Publication history: Received on 17 July 2022; revised on 12 September 2022; accepted on 14 September 2022

Article DOI: https://doi.org/10.30574/msabp.2022.7.1.0080

Abstract

The study was designed to evaluate the effect of boiling and microwave treatment methods on reducing the concentration of tannins in Soy bean (Sb), Cowpea (Cp) and Pigeon pea (Pg) legume grains (LGs). The raw samples of LGs were roasted in a microwave oven for 3 and 6 minutes at 120 °C Three (3) LG seeds weighing 30 gms were boiled for 30 and 60 minutes in water at 100 ^{o}C. Meals of Raw, boiled and microwaved Sb, Pp and Cp LGs were subjected to proximate analysis for levels of tannins using the Folin-Ciocalteu assay technique. Absorbance for each sample as indicator of tannin concentration was read from UV-VSI spectrophotometer. Tannic acid levels in micrograms for the three (3) samples were read from the standard curve and the tannin content was calculated as on dry matter basis. Raw samples showed no significant ($p<0.01$) difference in tannin content in Sb, Cp and Pp. Boiling the LGs in water for 30 minutes resulted in the reduction of tannin up to 46.6% Sb, 53.5% Pp and 42.5% Cp. Boiling for 60 minutes resulted in the reduction of tannins to 62.5 % Sb, 72.9% Pp and 75% Cp respectively. Microwaving the LGs for 3 minutes resulted in 46.7% Sb, 54.2 % Pp and 37.5% Cp reduction of tannin levels respectively. Microwaving for 6 minutes reduced tannins by 71.1% in Sb, 65.1% in Pp and 65% in Cp. No tannin significant ($p<0.05$) difference was observed for the effect of boiling and microwave treatment LGs.

Keywords: Tannin; Boiling; Microwave; Legume; Grains

1 Introduction

Economic challenges in developing countries have brought about an increasing demand for the use of grain legumes as alternative sources of proteins, energy and amino acids for human and livestock (Onwuke) [1] nutrition. The advent for the use of these traditional legumes comes on the back drop of low intake proteins due to scarcity of the conventional legumes.

Grain legumes such as Soybean (*Glycine max*), Pigeon peas (*Cajanus cajan*), Cowpeas (*Vigna unguiculata*) are increasingly being used for food and feed manufacturing in order to reduce capital expenses on nutrition. However, their usage is still limited because of uncertainty about the levels of anti-nutritional factors (ANF) naturally existing in them (Marek) [2]. Shimelis and Yogesh [3] defined ANF as factors that reduce nutrient utilization and restrict food intake of plant or plant products used as human food or animal feed. Among the ANFs are tannins, lectins, glycosides, phytates, alkaloids, trypsin and chymotrypsin inhibitors, amylase inhibitors and hem agglutinins (Chisowa et al) [4].

The ever-increasing prices of conventional feed due to the unstable ratio rates between American dollar and Zambian kwacha have led both commercial and subsistence farmers to exploit grain legumes that are available in their localities to produce food and feed. Despite the increase in the use of these legumes, the levels of ANF content still needs evaluation.

* Corresponding author: Donald Mudenda Chisowa
Department of Animal Science, School of Agriculture, Southern University, P. O Box 60293, Livingstone, Zambia.

On the other hand, Shimelis and Yogesh [3] added that the available data and information on the nutrient and ant-nutrient composition of the commonly used local food and feed do not cover the nutritional requirements of both humans and livestock. The report further states that this is due to the effects of variety, heredity, origin, climate, soil, processing methods, pesticides, fertilizers and composition of the food plant. However, Marek [2] contends that on account of numerous thermo-labile anti-nutritional factors contained in bean seeds, their dietary application depends on different forms and methods that may result in decreasing activity of anti-nutrients and increasing bioavailability of nutrients.

In view of the above, the treatment method used to prepare these legumes for food or feed is cardinal. There are several methods used such as roasting, soaking, boiling and microwave heating. Microwave heating, combining high yield and significant efficient, is one of the newest concepts in modern food processing (Regier and Schubert) [5] over the traditional boiling that has been in use for years. If nutrition in these legumes is to be efficiently utilized, the right method of processing for a particular grain needs to be used. Therefore, this research was conducted to compare the levels of ANF in three (3) different legume seeds subjected to different processing methods. This is aimed to reduce quantities of ANFs to harmless levels for human and livestock consumption. Although, this varies from one human to another and also from one livestock kind to another a general picture of safe levels should be established through research.

Djordje [6] observed that 'legumes play an important role in the traditional diets of many regions throughout the world.' Supporting the argument, Da Silva et al [7] confirmed the assertion by disclosing that Soybean (*Glycine max*) is a species of legume native to East Asia and widely grown for its edible seed which has numerous uses. He also stated that the plant is classified as an oil seed rather than a pulse by the UN Food and Agriculture Organization (FAO).

On the historical revelation of legumes, Da Silva et al [7] reported that in the humid tropics leguminous food crops are of special significance because of the low protein of the major staple crops consisting of root tuber and cereal. He further observed that scarcity of animal protein has warranted heavy reliance on plant protein in combating protein and calorie shortages in the tropics. Da Silva et al [7] further pointed out that the widely grown legume in Africa south of the Sahara desert is Cowpeas (*Vigna unguiculata*).

Djordje [6] disclosed that ''Soybean and its processed products have been acclaimed as healthy foods due to the high content of protein, essential amino acids, omeg-3 fatty acids, fat-soluble vitamins, polysaccharides and insoluble fiber.'' Besides the constituents mentioned Soybean also contains so-flavones that are of wide interest due to their beneficial effects on humans such as prevention of cancer, cardiovascular diseases, Osteoporosis and menopausal symptoms.

Mello et al [8] described Pigeon pea as ''a versatile crop cultivated mainly for its edible seeds which are high in protein.'' He mentions that its drought resistance makes it suitable for small scale farmers in the arid areas where rainfall is low. He also brings to light that it contains important B vitamins good for people living on subsistence diet. Mello et al [8] further claims that Pigeon peas are a good source of energy and protein in diets for monogastricts and an attractive alternative to imported oil seed meals and cereal grains. Nevertheless, Pigeon peas are said to have anti-nutritional factors as well. In a Journal of Food for Human Nutrition, it was reported that the concentration of protease inhibitors was present in Pigeon peas. The same study revealed that the levels were significantly ($p<0.01$) higher on some wild relatives of Pigeon peas. Protein digestibility of cooked pigeon meal remained low, suggesting that this could have been due to presence of certain compounds other than trypsin inhibitors alone. Onwuke [1] suggested that other toxicants present include lectins, oligosaccharides, and saponins, phenolic, tannins and phytates. He stressed the point that the proportion of these substances that may be relatively safe when consumed individually, however, can sometimes when taken together have serious and even fatal effects.

Onwuke [1] defined natural harmful effects as ''Natural toxicants or anti-nutritional factors.'' He pointed out toxicants such as hem agglutinins, trypsin inhibitors and chymotrypsin inhibitors. He further describes the inhibitors being responsible of lowering the digestibility of legumes. He also suggests that other factors such as tannins and phytates decrease the absorption of divalent metal ions in the intestine. Soybeans also contain hem agglutinins, a clotting promoting substance that causes red blood cells to clump together. Thomas warns that anti-nutritional factors are not completely deactivated during ordinary cooking.

Kemi et al [9] observed that several methods to reduce the levels of anti-nutritional factors have not been thoroughly investigated. He explains that these anti-nutrition factors form insoluble complexes with divalent ions Fe^{++} and Zn^{++} and thus making them unavailable for absorption. Onwuke [1] further mentioned of soaking in which the enzyme inhibitors end up concentrating in the liquid rather than in the card. Among the traditional processing methods, roasting of grains and cooking of vegetables were found to be more suitable mild treatments for preserving the tannin compound and its functional properties as opposed to soaking + cooking and blanching treatments (Kunyanga et al) [10]. Work by Reed [11] revealed that Proanthocyanidins are the most common type of tannin found in forage legumes. Problems in the analysis of tannins are that sample processing and drying decrease extraction and reactivity, suitable standards are unavailable, and quantitative analytical methods are poorly correlated with enzyme inhibition, protein precipitation, and nutritional effects

Vellingiri and Hans [12] further reported that the bioactive compounds were drastically reduced during soaking in tamarind solution + cooking as well as soaking in alkaline solution + cooking, and thus these treatments were considered to be more aggressive practices. Open-pan roasting also demonstrated a significant reduction of total free phenolics, tannins and moderate loss of L-Dopa and phytic acid.

1.1 Statement of the purpose

Despite their rich nutritive qualities, legumes are still underutilized; this fact is largely because of lack of knowledge of their nutritive potential and the presence of ubiquitous natural compounds capable of precipitating harmful effects in both humans and animals. The commonly used processing methods for mitigating the levels of anti-nutritional factors has been roasting and soaking. Other methods such as boiling and autoclaving have not been evaluated for their effect in mitigating the negative effects of anti-nutritional factors. It was against this background that this research was conducted.

Aim

To evaluate the effect of boiling and microwave treatment methods on tannin concentration in legume grains.

Objective

To determine the concentration of tannins in raw, boiled and autoclaved legume grains.

2 Material and methods

2.1 Research Site

The study was conducted in Monze District in the Southern Province of Zambia from February 2014 to March 2014. Thestudyareawas located between 14o 11‘S latitude and 33o 46’E longitude. The area lay 1100m above sea level with a mean annual temperature and mean annual rainfall of 33oC and 800- 1000 mm respectively.

2.2 Methodology

White Pigeon pea (*Cajanus cajan*), brown Cowpea seed (*Vigna unguiculata*) and cream white Soybean (*Glycine max*) were sourced and purchased from local peasant farmers of Monze and were used for the research. The process involved boiling and autoclaving the legume grains to mitigate the effects of tannins. The boiled and autoclaved legume grains were ground into powder using a hammer mill. The effects of boiling and autoclaving processing methods in eliminating anti-nutritional factors in the three (3) legumes were evaluated. Three (3) samples of powder for each legume were subjected to tannin analysis using proximate analysis. These samples were identified with labels R1, R2 and R3.

2.3 Research Design and Data Collection

Three (3) treatments of raw, boiled and autoclaved powder of Soybean, Pigeon pea and Cowpea each replicated three (3) times. Samples of Soybean, Pigeon pea and Cowpea each weighing 30 gms arranged in a Completely Randomised Design (CRD) were each sent to the University of Zambia for proximate analysis to determine levels of tannins using mechanical devices particularly the UV-VSI Spectrophotometer. Tannin content was analysed using the Folin-Ciocalteu assay technique in the Nutrition laboratory of the University of Zambia. Absorbance for each sample as indicator of tannin concentration was read from a spectrophotometer. Tannic acid levels in micrograms for the three samples were read from the standard curve and the tannin content was calculated as percentage on dry matter basis.

2.4 Processing of Legume Grains

2.4.1 Microwave roasting

The raw samples of legume grains were roasted in a Microwave oven for 3 and 6 minutes at 120^{o}C to a brownish colour to a level avoiding maillard reaction since proteins are heat labile. The grains were then removed from the oven air cooled and then ground to powder. Meal of the three (3) roasted replicates of ground Cowpeas, Pigeon pea and Soybean weighing 30g each were also sent to the University of Zambia for proximate analysis.

2.4.2 Boiling

Three (3) butches of each grain seeds weighing 30g were boiled for 30 and 60 minutes in water at 100 °C. The seeds were then recovered and water drained then, the seeds were sun dried. The sun-dried seed from each legume were powdered in a mill and sieved through a domestic mesh sieve. Ground samples of three (3) replicates of Cowpea, Pigeon pea and Soybean weighing 30g were sent for proximate analysis.

2.4.2.1 2.4.3 Statistics model

$$Y_{ijk} = \mu + l_i + m_j + d_k + (lm)_{ij} + (ld)_{ik} + (md)_{jk} + (lmd)_{ijk} + \varepsilon_{ijk}$$

Where; μ = overall mean
l_i =effect of i^{th} legume grain
m_j =effect of j^{th} treatment method
d_k =effect of k^{th} degree of treatment
$(lm)_{ij}$ = effect of two way interaction of i^{th} grain type and j^{th} method
$(ld)_{ik}$ = effect of two way interaction of i^{th} grain type and k^{th} treatment degree
$(md)_{jk}$ = effect of two way interaction of j^{th} method and k^{th} degree treatment degree
$(lmd)_{ijk}$ = effect of three way interaction of i^{th} grain type, j^{th} method and k^{th} treatment degree
ε_{ijk} = random error component

2.5 Data Collection

Data pertaining to tannin concentration in mg/g was recorded for each sample as indicated on the UV-VSI Spectrophotometer.

2.6 Data Analysis

Data recorded during proximate analysis was subjected to statistical analysis manually using the Statistical Analysis System (SAS). Treatment means were compared using F-test at $P < 0.01$ level of confidence.

3 Results

3.1 Tannin Levels in Raw Legume Grains

The results of tannin content of raw, boiled and autoclaved samples of the three (3) legume grains (Soybean, Cowpea and Pigeon pea) are shown in Tables 1 and 2. Raw samples indicated numerically but not significantly ($p<0.01$) higher tannin content in Soybean than in either Cowpea or Pigeon pea. The analysis revealed that tannin content was numerically lowest in raw Pigeon pea.

3.2 Tannin Levels in Raw, Boiled and Autoclaved Legume Grain Samples

Boiling the legume grains in water for 30 minutes resulted in the reduction of tannin up to 46.6% Soybean, 53.5% Pigeon pea and 42.5% Cowpea. Boiling for 60 minutes resulted in the reduction of tannins to 62.5% Soybean, 72.9% Pigeon pea and 75% Cowpea respectively (Table 1). Autoclaving the same legume grains for 3 minutes resulted in 46.7% Soybean, 54.2% Pigeon pea and 37.5% Cowpea tannin reduction. Autoclaving for 6 minutes resulted in 71.1% in Soybean, 65.1% Pigeon pea, and 65% in Cowpea tannin reduction (Table 1).

Table 1 Effect of microwave and boiling on tannin concentration (Dry Weight Basis) (mg/g)

Treatment	Legume type	Duration	R1	R2	R3	Total	Mean
Boiling	Soybean	30 min	0.22	0.27	0.24	0.73	0.24
		60 min	0.14	0.16	0. 15	0.45	0.15
	Pigeon Pea	30 min	0.22	0.20	0.18	0.60	0.20
		60 min	0.14	0.13	0.12	0.39	0.13
	Cow Pea	30 min	0.26	0.21	0.22	0.69	0.23
		60 min	0.06	0.12	0.12	0.30	0.10
Microwave	Soybean	3 min	0.23	0.25	0.24	0.72	0.24
		6 min	0.13	0.12	0.14	0.39	0.13
	Pigeon Pea	3 min	0.23	0.22	0.21	0.66	0.22
		6 min	0.15	0.16	0.15	0.45	0.15
	Cow Pea	3 min	0.25	0.25	0.25	0.75	0.25
		6 min	0.15	0.14	0.13	0.42	0.14

Table 2 Effects of boiling and micro-waving on tannin concentration in Soybean, Pigeon pea and Cowpeas

Treatments	Legumes grains		Tannin levels (mg/g)	Reduction %
Raw	Soybean		0.45^{a}	-
	Pigeon pea		0.43^{a}	-
	Cowpea		0.40^{a}	-
Boiling	Soybean	(30 min)	0.24^{a}	46.6
		(60 min)	0.15^{b}	62.5
	Pigeon pea	(30 min)	0.20^{a}	53.5
		(60 min)	0.13^{b}	72.9
	Cowpea	(30 min)	0.23^{a}	42.5
		(60 min)	0.10^{b}	75.0
Micro waving	Soybean	(3 min)	0.24^{a}	46.7
		(6 min)	0.13^{b}	71.1
	Pigeon pea	(3 min)	0.22^{a}	54.2
		(6 min)	0.15^{b}	65.1
	Cowpea	(3 min)	0.25^{a}	37.5
		(6 min)	0.14^{b}	65.0

^{ab}Means with different superscripts differ significantly($p<0.05$)

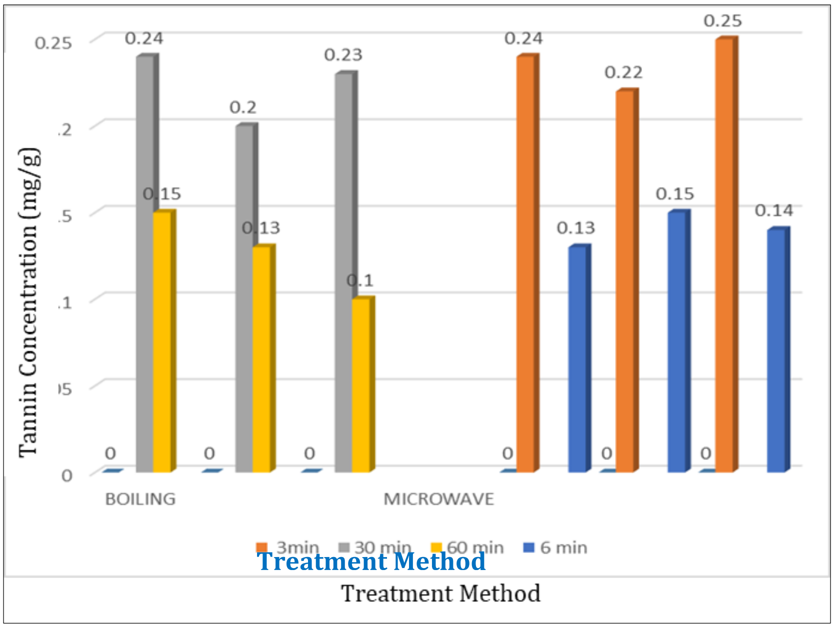

Figure 1 Effect of microwave and boiling treatment on tannin concentration

Table 3 Effect of tannin concentration on absorbance

Sample #	Conc(x) (mg/g)	Absorbance(y) (nm)
1	0.0	0.01
2	0.2	0.11
3	0.4	0.23
4	0.6	0.37
5	0.8	0.47
6	1.0	0.65

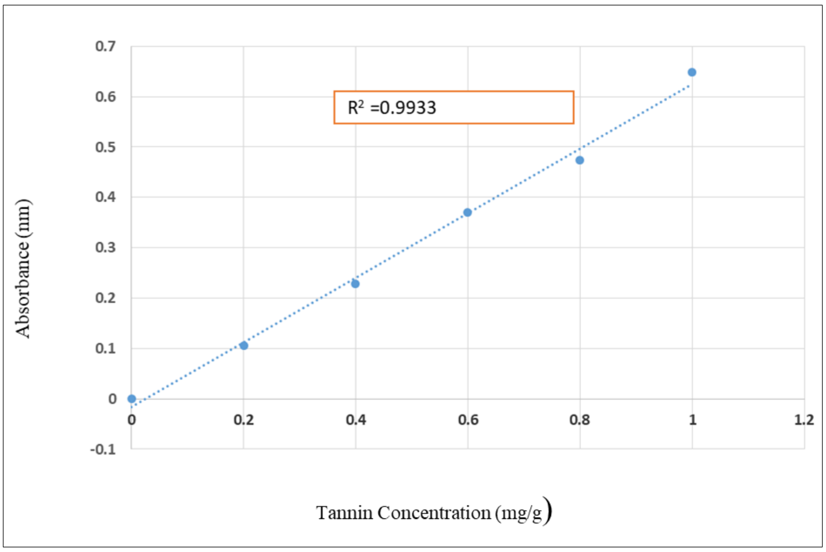

Figure 2 Beer Lambert Plot

Table 4 Effect of boiling period on tannin concentration in legume grains

TREATMENT	MEAN($\bar{y}$)	SD	SEM
30 min	47.5	±13.56	5.53
60 min	70.1		

Table 5 Effect of autoclaving period on tannin concentration in legume grains

Treatment	Mean($\bar{y}$)	SD	SEM
3 min	46.1	±12.83	5.24
6 min	67.1		

Table 6 Effect of boiling and autoclaving on tannin concentration in legume grains

Treatment	Mean($\bar{y}$)	SD	SEM
Boiling	58.9	±4.18	1.71
Autoclaving	56.6		

4 Discussion

In this study, there were no significant differences between boiling and micro waving as processing method to reduce the tannin levels in soybean, pigeon pea and cow pea grains. However, significant variations in tannin levels reduction were noticed between legumes at different times within treatments.

Mean values for tannin levels under both treatment methods and treatment levels did not differ significantly ($p<0.01$). Similarly, response of all legume types to the two (2) treatment methods did not show any significant differences as shown in Table 2. No significant differences were observed within and between treatment methods on tannin content. Results of the current study are at variance with those reported by Chisowa et al [4] who used roasting as a treatment method of legume grains for reducing tannins. The variation in the results can be explained in terms of the varieties used, climatic conditions and soil types in which the legumes are grown. These workers indicated significantly ($p<0.05$) higher tannin concentration in roasted Cowpea than in Soybean. The results of reduction in tannin concentration in Cowpea observed in this study are in consonance with findings of Adegumwa et al [13] who reported that tannin content in boiled Cowpea ranged between 0.13 to1.85 mg/100g.

The reduction in tannin content during boiling was as a result of the fact that tannins are polyphenols and all polyphenols are water soluble in nature. Therefore, the reduction in tannin content may be attributed to leaching out of phenols into the medium due to the influence of the concentration gradient ((Djordje) [6].

Micro wave treatment mean values for tannin levels for all legumes also did not show any significant differences as shown in Table 2. Similarly numerical differences were observed between time allocations though they were not significantly ($p<0.05$) different.

Autoclaving the legume grains for longer than 3 minutes decreases the tannin content to at least 65%. According to Singh [14] tannins are mostly found in the seed coat, therefore, the roasting and subsequent de-hulling the seed coat must have contributed to the reduction of the tannin levels in the grains.

The microwave heating, combining high yield and significant efficiency is one of newest concepts in modern food processing (Regier & Schubert) [5]. A drawback of Microwave processing of raw food material including processed bean seeds is the risk of reducing the nutritional value of the product. Therefore, the study suggests further research to investigate the effects this study may have on the nutrient values.

5 Conclusion

It was established that increasing boiling and micro waving time of legume grains progressively decreased anti-nutritional factors particularly tannin concentration in soybean, pigeon pea and cowpea seeds. Though effects of boiling and autoclaving did not differ significantly ($p<0.05$) there were numerical differences. Other higher treatment levels should be tried.

The study indicated that legume boiling treatment of 60 min treatment duration was numerically but not significantly ($p<0.05$) more effective in reducing tannin concentration in all legume types. A similar trend was observed in autoclaving where the 6min treatment was numerically effective than the 3min in all samples. Results of the current study point to the potential of other longer boiling and autoclaving durations than the ones used in this study in reducing tannin concentrations. The study has indicated a positive correlation between tannin concentration and absorbance.

Compliance with ethical standards

Acknowledgments

The author of this article is thankful to the laboratory technicians at the School Agriculture of the University of Zambia.

The publication of this article competes with methods of processing legumes used by some feed manufacturing industries in Zambia. Most feed manufacturing companies use Soybean as the main source of proteins in livestock feeds and may find results of this study providing competing alternatives in form of Cowpea and Pigeon pea.

Funding Statement

This work was supported by the Southern University Staff Development Department and the Southern Research Institute (SORI).

Data Availability

Readers can access the data used in the conclusions for this article by contacting the corresponding author through the following contact details: Email: mcmchisowa@yahoo.com.sg

References

[1] Onwuke GI. Soaking and boiling antinutritional factors in Pigeon peas. Journal of Food Processing and Preservation. 2006, 30(5): 76-81.

[2] Marek S, Miroslawa WJ and Malgorzata S. Determination of Trypsin Inhibitor activity of Microwave- heated Beans Seed using Bromocresole purple index (RCPI). Polish Journal of Food Nutrition Science. 2010, 60(4): 329–333.

[3] Shimelis AE and Yogesh J. Role of Antinutritional Factors in Food Industry. 1st ed. Adis Ababa (Ethiopia): Adis Ababa University Press. 2015.

[4] Chisowa DM, Mtimuni P, Phoya R and Chagunda M. Evaluation of Soybean (*Glycine max*), Cowpea (*Vigna unguiculata*) and Pigeon Pea (*Cajanus cajan*) as sole sources of proteins for growing rabbits---Access International Journal of Agricultural Sciences. 2013, 1(5): 57-67.

[5] Regier M and Schubert H. Microwave Processing, in: Thermal Technologies in food processing (ed. p. Richardson) pp 178-204. Cambridge, (England); Woodhead publishing Limited and CRC Press LLC. 2001.

[6] Djordje M, Jelena C and Jegor M. Polyphenol content and antioxidant properties of colored soybean seeds from central Europe. Journal of Medicinal Food. 2012, 15(1): 89-95

[7] Da Silva CA, Santos da CD, Junior TLD, Da Silva PB, Dos Santos CR and Siviero A. Cowpea: A Strategic Legume Species for Food Security and Health In book: Legume Seed Nutraceutical Research. Rio de Janeiro (Brazil): Intecopen; 2018. Available from (pp.https://www.intechopen.com/online-first/cowpea-a-strategic-legume-species-for-food-security-and-health).

[8] Mello D. Growing pigeon peas, an incredibly versatile permaculture plant. London (UK): CAB International Waillnfordoxon ox 108; 2013.

[9] Kemi EA and Fabiyi EF. Effect of Processing Methods on Some Antinutritional Factors in Legume Seeds for Poultry Feeding. International Journal of Poultry Science. 2010, 9(10): 98-103.

[10] Kunyanga CN, Imungi KJ, Okoth M, Momanyi C, Biesalski HK and Vellingiri V. Antioxidant and Antidiabetic Properties of Condensed Tannins in Acetonic Extract of Selected Raw and Processed Indigenous Food Ingredients from Kenya. Journal of Food Science. 2011, 76(4): 105-195.17

[11] Reed JD. Nutritional Toxicology of Tannins and Related Polyphenols in Forage Legumes' Department of Meat and Animal Science. Wisconsin (US). Journal of Animal Science. 1995, 73(5): 1516-28.

[12] Vellingiri V and Hans KB. Effect of certain indigenous processing methods on the bioactive compounds of ten different wild type legume grains. Journal of Food Science Technology. 2012, 49(6): 673–684.

[13] Adegunwa MO, Bakare HA, Alamu EO and Abiodun OK. Processing Effects on Chemical, Functional and Pasting Properties of Cowpea Flour from Different Varieties. Nigerian Food Journal. 2012, 30(1): 123-134.

[14] Singh U. Methods for Dehulling of Pulses: A Critical Appraisal. Journal of Food Science Technology. 1995, 32(2): 81-93.