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Proximate and Anti-Nutritional Analyses of Okra Seeds (Silver Queen, Star of David, and Clemson Spineless)

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Abstract:
This study evaluated the nutritional qualities in seeds of three varieties of okra (Silver Queen, Star of David, and Clemson Spineless) that are commonly cultivated in Makurdi, North Central Nigeria. Grand mean nutritional composition in okra fruits showed that carbohydrate was the highest class of food (43.7%) followed by protein (22.7%) and moisture (10.3%), fiber (9.2%), ash (7.2%) and lipid (6.9%). Result indicated that Silver Queen variety had the highest protein (22.93%), carbohydrate (45.57%), fiber (11.1%) and moisture (8.3%) while Clemson Spineless had the highest ash (7.07%) and lipid content (7.03%), although varietal differences are insignificant ($P>0.05$). Oxalate (0.51-0.88mg/100g) and cyanide (0.09-1.28mg/100g) were present as anti-nutrients but in very low concentration with insignificant varietal differences ($P>0.05$). Phytic acid content was the highest anti-nutrient quantified and it differed significantly among the varieties ($P<0.05$). Phytic acid was 49 times higher than oxalate and 59 times higher than cyanide in okra seeds tested. Phytic acid was lowest in Silver Queen (46.7mg/100g) while the highest amount was found in Clemson spineless (64.8mg/100g). Pearson's correlation showed negative coefficients between phytic acid and all nutrients except carbohydrate (0.840). From these findings, it could be deduced that Silver Queen variety was the best variety of nutritional values being high in carbohydrate, protein, fiber and moisture together with minimal level of anti-nutrients. Breeding efforts are needed to improve the nutrition of okra and reduce the anti-nutrients. This information is important to consumers in the quest to attaining national food security.

Keywords: Okra, Nutrition, Anti-nutrition, Breeding, Food security

INTRODUCTION

Okra fruit (*Abelmoschus esculentus* (L) Moench) popularly known as lady's finger is of the family Malvacea. It is native to Africa and therefore a popular vegetable crop in the tropical and subtropical regions cultivated in about 277,000 hectares of land in Nigeria (Ekwu and Nwokwu, 2013). Okra is one of the best soup condiments known so far and it is consumed in almost every home in Nigeria due to the ease of preparation and cost effectiveness. It contains quality nutritional properties such as water, protein, fat carbohydrate, fibre, ash and oil (Akinfasoye and Nwanguma, 2015). The fruit is a rich source of vitamin while the slimy mucilage has been reported to possess high carbohydrate contents and other medicinal values (Sale *et al.*, 2015). The crop was described as a multipurpose vegetable fruit crop (Dandena, 2012; Fekadu *et al.*, 2015).

The fruit has the potentials to help achieve food security in the vegetable segment due its low cost, ease of cultivation and preparation (Sanni *et al.*, 2015; Fagwalawa and Yahaya, 2016). However, it is under-utilized in many parts of Nigeria. Nutritional studies done so far focused on the fruits alone while the seed contents that form a bulky part is neglected (Habtamu *et al.*, 2014; Sale *et al.*, 2015) There are sketchy pieces of information on the nutritional values of the okra seeds. It is also uncertain if the okra varieties are devoid of toxic anti-nutrients that pose dangers to human health (Sale *et al.*, 2015; Arapitsas, 2018). Therefore, there is need to investigate three popular okra varieties (Silver Queen, Star of David and Clemson Spineless) sold in Makudi markets, Benue State, for their comparative nutritional values. The aim of the study was to investigate the proximate composition and determine the quantity of three anti-nutrients (cyanide, phytic acid and oxalate) in okra seeds. The specific objectives were to: determine the percentage composition of carbohydrate, protein, lipid, fiber, ash, moisture, oxalate, phytic acid and cyanide present in the three okra varieties; and also determine the relationship among the nutrients and anti-nutrients.

MATERIALS AND METHODS

The Study Area

This study was conducted in the General Biology Laboratory, College of Biological Sciences, Joseph Sarwuan Tarkaa University Makurdi, Benue State.

Sample Collection, Identification and Preparation

Seeds were sourced from the seed bank germplasm of the Botany Department of the same institution and authenticated by Dr. J.O. Olasan as Clemson spineless, Star of David and Silver Queen. Exactly 500g of each sample, labeled as V1-V3, was pounded into powder form and tied separately and stored till further use

Proximate Analysis

Moisture Content:

Moisture content was determined using the oven dry method (Adetuyi *et al.*, 2011). It was calculated as: by:

$$\% \text{ Moisture} = \frac{w2-w3}{w2-w1}\ x \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where, w1= weight of empty moisture can (petri dish); w2= weight of can and sample before drying; w3= weight of can and sample after drying

Crude Protein:

The micro-kjeldahl titration method was used where samples were mixed with 10ml of concentrated tetraoxosulphate (vi) acid in a kjeldahl digestion flask (Simone, 2014). The total nitrogen was calculated and multiplied by a factor 6.25 to obtain the crude protein content as % Crude protein =%N6.25.

$$\% \text{ N2} = \frac{(100\text{x})\text{Nx14xVfxT}}{\text{w x 100 x VA}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

W= weight of the sample; N = Normality of filtrate ((H_2So_4) = 0.02N; V_F = Total volume of the digest = 100ml; V_A = Volume of the digest distilled

Determination of Fat:

Fat content was determined by the solvent extraction method using a soxlet apparatus (Adetuyi *et al.*, 2011). The weight of the fat (oil) extracted was expressed as percentage of the sample weight.

$$\% \text{ of fat} = \frac{w2-w1}{w1} x \frac{100}{1} \ldots\ldots (3)$$

W = weight of the sample; W_1 weight of empty extraction flask

Ash Content Determination:
Ash cntent was determined using the furnace incineration gravimetric method (Adetuyi *et al.*, 2011). Percentage ash content was determined as

$$\% \text{ Ash} = \frac{w2 - w3}{w2 - w1} x \frac{100}{1} \ldots\ldots (4)$$

W_1 = weight of the crucible; W_2 = weight of sample in crucible; W_3 = weight of crucible + ash

Determination of Crude Fiber:
This was determined by the Weende method **(**Adetuyi *et al.*, 2011**)** and calculated as

$$\% \text{ Crude fibre} = \frac{\text{loss in weight incinertion}}{\text{weight of sample}} x \frac{100}{1} = \frac{w2 - w3}{\text{weight of sample}} \ldots\ldots (5)$$

W_2 = weight of crucible sample after washing and drying in oven; W_3 = weight of crucible + sample ash

Determination of Carbohydrates:
Carbohydrates was determined by Nitrogen free extraction (NFE) method (Adetuyi *et al.*, 2011). The NFE was calculated as

% NFE = 100 - % (a+b+c+d+e) .... (6)

Where: a= protein; b= fat; c= fibre; d= ash; e= moisture

Determination of Anti-nutritional Factors in Okra Seed:
Concentration of all anti-nutrients in okra seed was determined in mg/100g using standard methods (Habtamu *et a.*, 2014). The oxalate content was calculated by taking 1ML of 0.05M $KMnO_4$ as equivalent to 2.2mg oxalate. Phytic phosphorus (Pp) was determined and the phytic acid content was calculated by multiplying the value of Pp by 3.55 where Fe equivalent=1.15x titer value and Pp= titer valuex1.19x1.95. Therefore, phytic acid =1.95x1.19x3.55x titer value. Alkaline picrate colour development was used in the determination of cyanide where absorbance was read at 450nm against blank.

Data Analysis
Data was analyzed on the Minitab 17.0 software using the following statistical tools: Chi Square test of dependency; One way ANOVA ($P \leq 0.05$) and Pearson's correlation.

RESULTS AND DICUSSION

Table 1 shows the proximate composition of three varieties of okra: Silver Queen, Star of David, and Clemson Spineless. Silver Queen had the highest protein (22.93%), carbohydrate (45.57%), fiber (11.1%) and moisture (8.3%) while Clemson Spineless had the highest ash (7.07%) and lipid content (7.03%) among the three varieties. The observed differences in a particular nutrient for all varieties are insignificant statistically (P>0.05), Grand mean nutritional composition in okra fruits showed that carbohydrate was the highest class of food (43.7%) followed by protein (22.7%). Others are moisture (10.3%), fiber (9.2%) and ash (7.2%) while lipid content was the lowest (6.9%) in the fruit (figure 1).

The nutritional compositions in three okra varieties reported in this work are in tandem with the available data in literature (Adetuyi *et al.,* 2011; Olujobi and Ayodele, 2013; Habtamu *et al.,* 2014; Arapitsas, 2018), thus confirming its dietary values. In this work, only the seed component was investigated for proximate and anti-nutrients as only dry seeds were available in preserved forms with known identities. This study has further supported claims that the seeds are of valuable nutritional qualities in okra fruits and that consumers should avoid discarding them during food preparation. The reported amount of carbohydrate of 44.7% being the highest followed by protein (22.7%), moisture (10.3%), fiber (9.2%), ash (7.2%) and lipid (6.9%) has shown that the seeds are much more valuable than the entire okra fruits based on previous nutritional analysis done on the fruits by some researchers (Khomsug *et al.,* 2010; Roy *et al.,* 2014; Habtamu *et al.,* 2014). It thus suggests that the okra seeds present a form of balanced nutritional diet that should be embraced to reduce the level of malnutrition in developing countries. The seeds may therefore be incorporated into the production of industrial feeds and food supplements for humans and livestock. The three varieties studied have therefore showcased quality nutritional values. However, the Silver Queen variety seems to be the best.

Table 2 presents the anti-nutritional factors (cyanide, oxalate, and phytic acid) content in three varieties of cowpea. Cyanide and oxalate contents were relatively low ranging from 0.09-1.28mg/100g in cyanide where Star of David had the maximum value while it was 0.51-0.88mg/100g in oxalate where Silver Queen had the maximum value though not significantly different from other varieties (P>0.05). Phytic acid content was the highest anti-nutrient quantified and it differed significantly among the varieties (P<0.05) where it was lowest in Silver Queen (46.7mg/100g) while the highest amount was found in Clemson spineless (64.8mg/100g). Figure 2 shows that the overall mean phytic acid of 62.3 mg/100g was 49 times higher than oxalate and 59 times higher than cyanide in okra

The anti-nutrients found in the okra seeds are important for discussion. All the values reported in this study are below the permissible limits of WHO (WHO, 2015) for anti-nutritional factors and therefore, the three varieties are healthy for consumption. Oxalate and cyanide were present but in negligible amount. These factors are part of the normal phytochemical compounds present in plants as products of metabolic biosynthetic pathways, although they interfere with absorption of nutrients leading to deficiencies in essential minerals (Khomsug *et al.*, 2010; Roy *et al.*, 2014). The most important of all the nutrients studied in this work is the phytic acid component being the highest quantified (about 49-59 times higher than the amount of oxalate and cyanide respectively in okra seeds), although it was below regulatory limits. Significant variation was also observed in the level of phytic acid among the three varieties and this may be due to genetic factors (Adiger *et al.*, 2011; Bisht *et al.*, 2015; Ford, 2018).

From all indications, there are needs to reduce the level of phytic acid in the seeds. This is important to ensure that the body absorbs the rich nutrients present in the seeds maximally without interference from phytic acid (Roy *et al.*, 2014). Although the mineral component was not included in this study, there are claims that phytic acid reduced the amount of minerals such as zinc and iron in foods needed by the body system (Roy *et al.*, 2014). This study found that the Silver Queen variety that came top in proximate analysis also emerged the best in the anti-nutritional study being the lowest in phytic acid content. It therefore suggests that this variety possibly possesses certain genes that drive the production of useful biochemical compounds as well as those that suppress the production of others. This is because the production of all forms of active ingredients and metabolites in plants are regulated by genes (Bisht *et al.*, 2015; Ford, 2018).

Ash had high positive correlation with Fibre (0.958), Protein (0.995), and Oxylate (0.697). Moisture had negative correlation with all variables except Ash) as obtained in Table 3. Fibre had a positive correlation with Ash (0.958), Protein (0.981), and Oxylate (0.873). Phytic acid had a negative correlation with all variables except Carbohydrate (0.840). It shows that carbohydrate level positively influences phytic acid level in okra seeds.

Therefore, the high amount of carbohydrate reported in this study may account for the escalated quantity of phytic acid. There are many options for breeders in this regard. The carbohydrate level may reduce or varieties that have low phytic acid content may be bred. This suggestion conforms with some reports where food crops are improved to reduce allergies (Siemonsmo, 2012; Singh and Oswalt, 2015).

The negative relationship established between other nutrients and phytic acid is a welcome development because when nutrients like protein, lipids, fiber and moisture are improved upon, the phytic acid level may reduce drastically. Okra seeds are rich sources of nutrients; hence consumers should not discard them in meal preparation. The Silver Queen variety should be popularized for cultivation and consumption for its additional values. Further studies are needed to evaluate all types of anti-nutritional factors in okra and other vegetables. Breeding efforts are needed to improve the nutrition of okra and reduce the anti-nutrients.

Table 1: Proximate composition in three varieties of okra

Varieties	Moisture (%)	Ash (%)	Fiber (%)	Lipid (%)	Protein (%)	Carbohydrate (%)
SQ	8.33±0.33	8.17±1.59	11.17±1.45	7.00±0.29	22.93±0.10	45.57±1.44
SD	11.33±0.17	6.40±0.76	8.00±0.29	6.70±0.15	22.52±0.06	43.15± 0.37
CS	11.17±0.60	7.07±0.34	8.33±0.17	7.03±0.09	22.64±0.06	39.51±0.58
Grand mean	10.28±0.97	7.21±0.52	9.17±1.01	6.910±0.105	22.69±0.12	42.74±0.99

Carbohydrate: χ^2 (Variety Vs Carbohydrate content) = 0.435, P=0.804 (P>0.05)
Moisture: χ2 (Variety Vs moisture content) = 0.55, P=0.758 (P>0.05)
Ash: χ2 (Variety Vs Ash content) = 0.22 P=0.895 (P>0.05)
Fibre: χ2 (Variety Vs fibre content) = 0.0663 P=0.718 (P>0.05)
Lipid: χ2 (Variety VsLipid content) = 0.01 P=0.995 (P>0.05)
Protein: χ2 (Variety Protein content) = 0.004, P=0.0998 (P>0.05)
SQ=Silver Queen, SD= Star of David; CS=Clemson Spineless

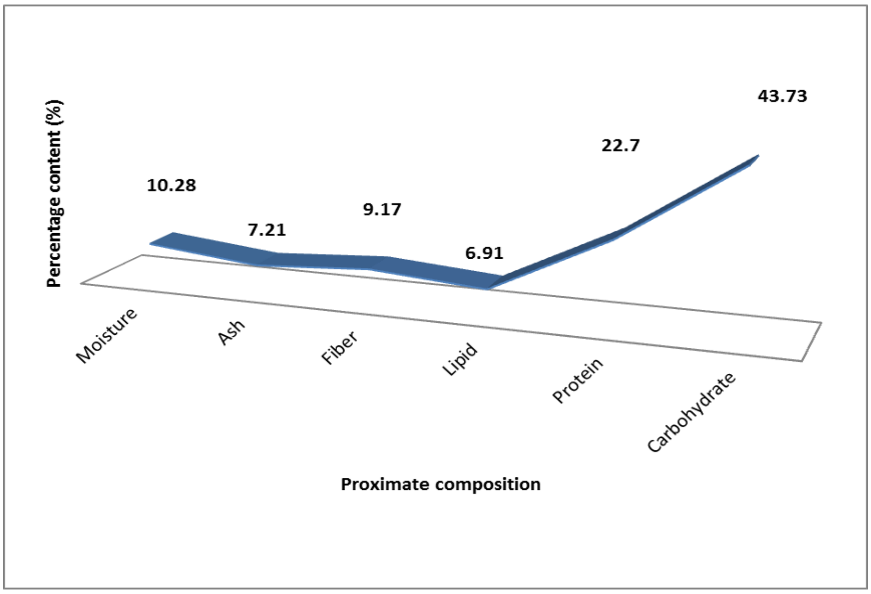

Figure 1: Graph showing the proximate composition as express in percentage

Table 2: Anti-nutritional factors in three varieties of Okro

Varieties	Cyanide (mg/100g)	Oxalate (mg/100g)	Phytic acid (mg/100g)
Silver queen	0.97 ^{b}±0.04	0.88 ^{a}±0.13	46.65 ^{c}±0.71
Star of David	1.28 ^{a}±0.01	0.66 ^{a}.±0.13	75.49^{a}±0.74
Clemson spineless	0.09 ^{b}±0.01	0.51 ^{a}±0.07	64.8 ^{b} ±0.74

F (Cyanide content) = 74.60, P= 0.00 (P<0.05)
F (Oxylate content) = 2.71, P=0.145 (P<0.05)
F (Phytic acid content) = 401.52, P=0.00 (P<0.05)
Means that do not share a letter are significantly different.

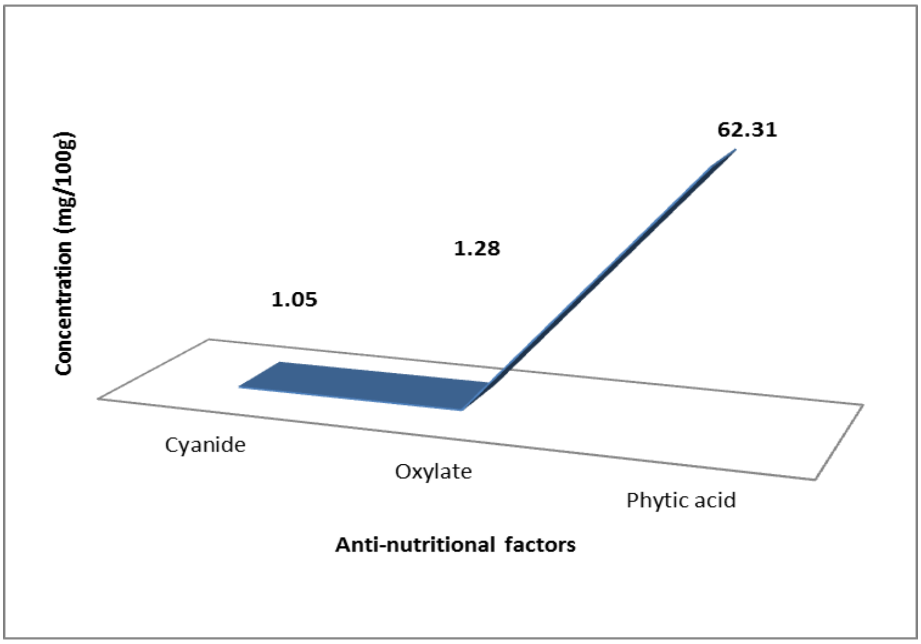

Figure 2: Anti-nutritional factors in three varieties of Okra

Table 3: Pearson's Correlation Matrix

	Ash	Moisture	Fibre	Lipid	Protein	Carbohydrate	Cyanide	Oxylate	Phytic acid
Ash	1								
Moisture	-0.941	1							
Fibre	0.958	-0.998	1						
Lipid	0.735	-0.462	0.511	1					
Protein	0.995	-0.969	0.981	0.667	1				
Carbohydrate	-0.845	0.613	-0.657	-0.984	-0.790	1			
Cyanide	-0.670	0.379	-0.430	-0.996	-0.596	0.963	1		
Oxylate	0.697	-0.899	0.873	0.027	0.763	-0.205	0.065	1	
Phytic acid	-1.000	0.944	-0.961	-0.729	-0.996	0.840	0.663	-0.704	1

CONCLUSION

Okra seeds are rich in balanced nutrients and therefore of high nutritional values. Although the three varieties have good nutritional qualities, the Silver Queen variety was the best being high in carbohydrate, protein, fiber and moisture together with minimal level of anti-nutrients. It is important to improve all the varieties for certain nutrients such as protein and lipids so as to reduce the phytic acid level based on the results obtained. This information is important to the breeders, the growers, consumers and the general public.

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