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COMPARATIVE EFFECTS OF THREE LEVELS OF INSECT INFESTATION ON SELECTED PROPERTIES OF FIVE NIGERIAN COWPEA (*Vigna unguiculasta* (L.) WALP) VARIETIES

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ABSTRACT
Effect of three insect infestation levels (25%, 50% and 75%) on selected properties of five Nigerian cowpea varieties (Ife brown, Iron beans, Isiocha, Patasco, and Sokoto white) were compared and studied. Infestation levels of each variety were obtained by counting and combining infested and un-infested seeds. Samples were analyzed for protein, total dietary fiber (TDF), blue value index (BVI), swelling index (SI), foaming capacity (FC) and foam stability (FS). Infestation at all levels significantly ($p<0.05$) reduced mean TDF (12%-21%), SI (3%-13%) and FS (22%-37%) of the cowpeas. Infestation also significantly ($p<0.05$) reduced protein (21%-24%) and FC (21%-41%) of the cowpeas while it significantly ($p<0.05$) increased BVI (49-80%). No significant difference ($p>0.05$) was observed between mean values of control and 25% infested samples for BVI and FC. No significant difference ($p>0.05$) was observed among mean values of 25%, 50% and 75% infested cowpeas for FS and between mean values of 25% and 50% infested cowpeas for protein. Infestation caused higher reduction in protein and FS of Iron beans and higher increase in BVI and decrease in FC of Isiocha and Sokoto white. Patasco was least affected by insect infestation in protein content, level of starch damage and foaming capacity; Iron was least affected in TDF and SI while Ife brown was the least affected in foaming stability. Un-infested samples were better in all properties evaluated and these varied among the varieties. Un-infested Sokoto possessed better foam properties, with 2.0g/g foam stability.

Key words: Cowpea varieties, infestation levels, blue value index, protein, Nigeria.

INTRODUCTION
Cowpea has been one of the most ancient and important food legumes in Africa, Asia and the Mediterranean countries for more than 3500 years (Gayan *et al*., 2006). It is the most important grain legume crop in Sub-Saharan Africa according to CGIAR (2017) and a major staple and source of inexpensive protein and other vital dietary components such as dietary fiber, calories, minerals, B-vitamins and essential fatty acids, especially among West Africans (National Research Council, 2006; Abudulai *et al.,* 2016; CGIAR, 2017; Kyei-Boahen *et al.,* 2017; Singh and Mukhi, 2017). Annually, about 12-15 million hectares of land is cultivated with cowpea worldwide with the Sub Saharan Africa accounting for the bulk of the total area of production (about 12 million hectares) and Nigeria responsible for about 1/3rd (4-5 million hectares) of total world production area. Nigeria is the largest producer and consumer of cowpea globally with an estimated annual production of 2-3 million tons or about 60% of the world's total annual production of about 5 million tons (Ajetomobi and Abiodun, 2010; African Agricultural Technology Foundation, 2012; Oyewale and Bamaiyi, 2013; Kamai *et al*., 2014; Kamara *et al*., 2016; Ahmad and Kiresur, 2016; CGIAR, 2017; FAO, 2016). In Nigeria, cowpea is mainly consumed in the dried seed form as porridge or in combination with foods such as cereals e.g. rice, or processed into paste which is used in making the popular relished 'akara' and 'moin-moin' (Oboh and Agu, 2010; Odejayi *et al*., 2014). It is also used as composite flour in the production of infant foods and baked and fried products such as bread, cake and chin-chin where they improve especially the protein quality of the products (Uzogara and Ofuya, 1992; Akubor, 2004; Oladunmoye *et al.,* 2010; Vilakati *et al.,* 2015).Unfortunately, cowpea is prone to heavy

field and post harvest infestation damage and this constitutes one of the major constraints to its optimal utilization as food as well as limits its contribution to food security (Kungu *et al*., 2003). Cowpea is attacked in the field where yield reductions of 20 to over 90% by the field insect pests such as *Megalurothrips sjostedti*, *Aphis craccivora, Helicoverpa armigera* and *Maruca vitrata* has been reported in Cameroun and Nigeria and in some other producing countries (Carlos, 2004; Ngakou *et al*., 2010; Muchero *et al*., 2010; Margam *et al*., 2011; Abudulai *et al*., 2016; Sangoyomi and Olufunmilola, 2016; Alale *et al.,* 2017; Kattula *et al*., 2017). Cowpea is further severely damaged in the store by the foremost cowpea storage pest, the cowpea weevil *Callosobruchus maculatus* Fab. Mylabridae, which bore hole into the seeds resulting in loss of food grain and quality.

The losses incurred by this weevil to cowpea in storage is reported to reach 100% sometimes, if left unattended, compelling many farmers in Africa, Asia and Southern America to dispose of their crops soon after harvest thus reducing an important protein and income source for their families (Sanon *et al*., 2010; Ajayi *et al.,* 2015; Oyeniyi *et al.,* 2015). Significant losses in weight, viability, protein content, essential amino acids, total fat, mineral matter, vitamins, soluble sugars, starch digestibility, emulsification, foam and viscosity properties and increase in free fatty acids and peroxides of insect-infested grains were reported (Ojimelukwe *et al*. 1999; Sallam, 2008; Odejayi, *et al*., 2014). Jood *et al*. (1993) reported deleterious effects on protein quality (such as biological value, net protein utilization and nitrogen absorption) and growth performance of study rats fed insect infested sorghum grains. The activity of insect pests in stored grains was also found to leave behind sloughs, frass and other secretions and to raise the products temperature and humidity which all predispose the grains to bacterial and fungi contamination especially aflatoxin poisoning, suggesting the potential danger infested cowpea can pose to the consumer in addition to losses in nutrients, functional properties and unpleasant sensory qualities (Carlos, 2004; Sallam, 2008; Odejayi, *et al*., 2014; Odejayi and Aina, 2016).

It is still common place to find both moderately to severely infested cowpeas in Nigerian markets as consumers tend to ignore the consequences of infestation on the nutrient quality if the physical appearance, organoleptic and/or functional properties have not deteriorated appreciably (Ojimelukwe *et al*., 1999). Many researchers had earlier worked on cowpea and generally compared infested cowpeas with un-infested cowpeas. There is paucity of information on the effect of infestation on the qualities (such as total dietary fiber (TDF) and blue value index (BVI)) of cowpeas at different degrees of infestation. This is necessary to determine the acceptability and usefulness of the infested cowpeas at different levels of infestation. This study was therefore carried out to evaluate the effect of different infestation levels on nutritional and functional properties of five Nigerian cowpea varieties.

MATERIALS AND METHODS

Seeds of Ife-brown, Iron, Isiocha, Patasco and Sokoto white cowpea varieties (Figures 1-5) used for this study were purchased from Eke Ukwu Market in Owerri, Eastern Nigeria.

Preparation of samples

Seeds of each variety were sorted into infested and un-infested seeds. Un-infested and infested seeds of each variety were then combined by counting on 1000 seeds basis to obtain samples of 0%, 25%, 50% and 75% levels of infestation. Table 1 shows how the samples were generated. Each sample was de-hulled, winnowed and milled into flour using a hammer mill. The samples were packaged in low density polyethylene wraps and stored in an air-tight container until used for analysis.

Crude protein determination

The Kjeldahl method described by A.O.A.C (2010) was used. Each sample (0.1 g) was mixed with 20 ml of concentrated H_2SO_4 in a Kjeldahl flask. A small amount of a mixture of copper sulphate and anhydrous sodium sulphate catalyst was added to the flask and the mixture heated inside a fume cupboard. The digest was transferred into a 100 ml volume flask and diluted to mark with distilled water. An aliquot of the digest (20 ml) was mixed with equal volume of 45% NaOH solution in a semi-micro Kjeldhal distillation apparatus. The mixture was distilled and the distillate collected into 10 ml of 4% boric acid solution containing 3 drops of mixed indicator, methyl red and bromocressol green. A total of 30 ml distillate was collected and titrated against 0.01N H_2SO_4 solution. The percentage nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein content.

$$N\% = \frac{(100 \times n \times 14 \times VF)\ T}{100 \times Va}$$

Where N% = percent nitrogen content; n = normality of acid used; VF= total volume of the digest= 100ml; T= titre value; Va= aliquot volume distilled.

Total dietary fibre determination
Total dietary fiber (TDF) was determined by the method of AOAC (2010). One gram (1 g) weight of each sample was mixed with 10 ml of distilled water in a beaker and gelatinized in a water bath (whilst stirring continuously) followed by digestion or incubation with termamyl enzyme (pH 6, 100°C, 30 min). At the end of termamyl incubation, the pH of the sample was adjusted to 7.5 with 1.25% NaOH. The temperature of the sample was adjusted to 60°C, protease was added and sample incubated at this temperature for 30 min. The sample was subsequently incubated at 60°C with amyloglucosidase for 30 min after adjustment to pH 4.5 with acetic acid. The total dietary fiber was precipitated with four volumes of ethanol, followed by filtration and washing of the residue with ethanol and acetone, and drying in an oven at 100°C. The total dietary fiber was calculated as

$$TDF = \frac{\text{weight of residue after drying}}{\text{weight of sample}} \times 100$$

Swelling index determination
Swelling index was determined by the method of Ukpabi and Ndimele (1990). Three gram (3 g) portions of each flour sample were transferred into clean, dry graduated (50 ml) cylinders. The flour samples were gently leveled and the volumes noted. Distilled water (30 ml) was added to each sample; the cylinder was swirled and allowed to stand for 60 minutes. The swelling power of each flour sample was calculated as

$$\text{Swelling Index} = \frac{\text{volume of sample after swelling}}{\text{original volume of sample}}$$

Determination of foaming capacity and foam stability
Foaming capacity and foam stability were determined as described by Lawhen *et al.* (1972). One gram (1g) weight of each flour sample was mixed with 10 ml distilled water using a magnetic stirrer (PHYWE) at 10 Ruhrer speed at room temperature for 5 min. The whipped mixture was transferred into 25ml graduated cylinder and the foam volume read after 30 sec. The volume of foam at 30 sec was regarded as foam capacity and the volume after 30 min standing as foam stability.

Blue value index determination
BVI was determined by the method of Kawabata *et al.* (1984). One gram (1g) weight of each flour sample was dispersed in 10 ml distilled water. The dispersion was stirred occasionally for 30 min, and then filtered through What-man No. 42 filter paper. Two ml of the filtrate was transferred into a conical flask and 4 drops of phenolphthalein was added and titrated with 0.1N iodine solution. The titre value was recorded and BVI calculated as

$$BVI\ (ppm) = \{(V_D/V_A)(X/M_f)(N/1000)\} \times 10^6$$

Where V_D = total volume of dispersion; V_A= volume of aliquot used in titration;
X = titre value; M_f = weight of flour sample used; N = normality of iodine solution used

Statistical analysis:
Data obtained from the study were computed into means ± SD. Analysis of variance (ANOVA) was used to detect any significant difference due to insect infestation. Where significant differences exited, Fishers Least Significant Difference (F-LSD) was used to separate the means. Significant difference in sample means was accepted at $p<0.05$.

RESULTS AND DISCUSSION

Protein content: Table 2 shows the protein content of the un-infested and infested cowpeas. The mean protein content of un-infested samples, 19.97% (varying between 15.31% in Isiocha and 22.75% in Ife br own and Iron) did not differ significantly ($P>0.05$) from the mean protein content of 75% infested cowpeas (17.68%) but differed significantly ($P<0.05$) from the mean protein content of 25% (15.73%) and 50% (15.18%) infested cowpeas. The mean protein content of 25%, 50% and 75% infested cowpeas was not significantly ($P>0.05$) different. Insect infestation significantly ($P<0.05$) reduced mean protein content of the cowpeas by 21-24% with 50% infestation causing the highest reduction of 24%. Reduction in protein content due to infestation was highest in Iron (50%) and least in patasco (5%) (Table 2). The protein content of un-infested Ife brown (22.75%), Iron (22.75%) and Patasco (21.56%) agreed with the range (22-25%) recorded for un-infested cowpeas (Ihekoronye and Ngoddy, 1985). Depletion in protein content of infested cowpeas was similarly observed by Ojimelukwe *et al.* (1999). Ojimelukwe *et al.* (1999) reported a decrease of 19% and 28% in protein content of Kano white and Ife brown varieties, respectively which agreed with 21-24% decrease in protein content observed in this study. Depletion in protein content of infested samples was attributed to utilization of the protein nutrient by the insects (Ojimelukwe *et al.,* 1999). The increase in mean protein content of 75% infested cowpeas cannot be a true increase in protein content. Jood *et al.* (1993) noted that no matter the direction of change in the nitrogen content of infested grains, the true protein content of such grains decrease and the real nutritional value of protein is not reflected in chemical analysis of infested cowpeas because of a number of factors like presence of insect excreta and body fragments among others. Biological evaluation of protein quality is most desirable in such situation according to Jood *et al.* (1993).

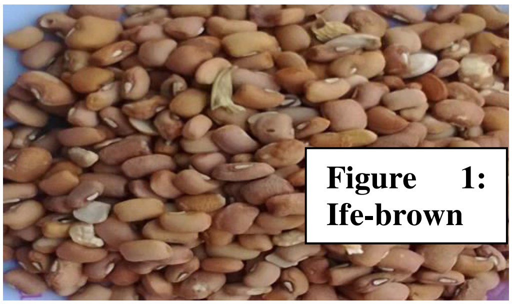

Figure 1: Ife-brown

Figure 2: Iron beans

Figure 3: Patasco

Figure 4: Isiocha

Figure 5: Sokoto white

Figures 1-5: Nigerian Cowpea Varieties

Total dietary fibre:

The total dietary fiber of the un-infested and infested cowpeas is presented in Table 2. The TDF of un-infested cowpeas varied from 34% in Patasco to 49% in Iron beans and Isiocha with a mean TDF value of 42.60% which was significantly different (P<0.05) from the mean TDF value (33.6-37.6%) of the infested samples. Infestation at all levels significantly (p<0.05) reduced mean TDF of the cowpeas by 12-21% with 75% infestation causing the highest reduction. Highest reduction in TDF (35%) occurred in Isiocha while the least reduction in TDF occurred in iron beans (4%) at 75% infestation level. The TDF of the un-infested cowpeas (34-49%) was in close agreement with the range of 20.9 to 46.9 g/100 g TDF reported for a number of legumes including soybean, peanut and cowpea among others by Mallillin *et al.* (2008). Decrease in TDF in the infested cowpea samples was attributed to feeding on the soluble portion of TDF by the insects. Lon (2005) reported that all animals use nearly all of the soluble fiber ingested. The high TDF recorded for the un-infested cowpeas makes them of considerable physiological and health benefits in terms of regulation of blood sugar, cholesterol lowering, and prevention against heart disease, diabetes and certain forms of cancer among other physiological benefits (Anderson *et al.*, 2009).

Blue value index (BVI):

The BVI of the un-infested and infested cowpeas is presented in Table 2. The BVI of un-infested samples varied from 37.50 ppm in Iron and Sokoto to 51.25ppm in Ife brown. Infestation significantly (P<0.05) increased mean BVI of the cowpeas from 20% at 25% infestation level to 80% at 75% infestation level, indicating significant (P<0.05) starch damage of the cowpeas by infestation. The mean BVI of control samples did not differ significantly (P>0.05) from that of 25% infested cowpeas (Table 2). However, at 25% infestation level, BVI increased by 32% in Isiocha to 53% in iron beans but by only 2% in Ife brown and 18% in patascco. Significant starch damage at 25% level of infestation may therefore be said to have occurred in all the varieties except in Ife brown. Generally, infestation caused a higher increase in the BVI of Isiocha and Sokoto, causing over 100% increase in their BVI, respectively. This may indicate more fragility of starch granules than the other varieties (Mbofung, 2006). BVI values for un-infested cowpea samples suggest thermal and mechanical damage of starch granules which according to Ezema (1989) occur during grinding or milling into flour.

Blue value index is used to represent the degree of starch damage or fragility (Mbofung, 2006) and to correlate the amount of free starch from mechanically damaged cells (Lulai, 1983); the higher the starch damage or damage of starch granules, the higher the BVI. The high increase (20-80%) in BVI of the infested cowpeas showed that infestation severely damaged their starch granules and consequently reduced their SI which according to Ezema (1989) depend partly on the extent of starch damage. The higher reduction in BVI recorded for Isiocha and Sokoto shows the extent infestation can limit their usefulness in making the relished *akara* for which they are most suitable and used for traditionally.

Swelling index (SI)

The swelling index of the un-infested and infested cowpeas is presented in Table 2. The SI of un-infested samples varied from 1.29 ml in Patasco to 1.61 ml in Ife brown with a mean SI value of 1.42ml which differed significantly ($P<0.05$) from the mean SI value for 25% (1.38 ml), 50% (1.31 ml) and 75% (1.23 ml) infested samples. Infestation at all levels significantly ($P<0.05$) reduced the mean SI of the cowpeas (3-13%) and 75% infestation caused the highest reduction in SI. Infestation least affected the swelling index of iron beans, causing only 1-6% reduction in its SI while up to 14-16% reduction was recorded in the other varieties. Decrease in the swelling index of the infested cowpeas was attributed to reduction in protein, TDF and starch damage by infestation. Enwere (1985) reported that proteins in flour contributed to swelling to about 3.75 times its original volume while Ezema (1989) reported that the extent of swelling in the presence of water depends on extent of starch damage and carbohydrates such as pectins, hemicelluloses and celluloses. Swelling of starch granules result in increased viscosity as a result of gelatinization and this phenomenon according to Enwere (1985) is the basis for the production of cowpea products '*akara'* and '*moin-moin*' where the starch in cowpea flour swell when heated in excess of water above $60^{o}C$ to about 6.13 times their original volume by initially absorbing water, gelatinizing and then swelling. Infested cowpea seeds would therefore not be suitable for making *moin-moin* and *akara*.

Foaming capacity (FC) and foam stability (FS):

About 13% to 41% reduction in FC was observed for the cowpeas with 75% infestation causing the highest decrease in FC. The FC of patasco was not apparently affected by infestation, 25% infestation did not affect Ife-brown while 25 and 50% infestation did not affect Iron.

Infestation reduced FC of Isiocha and Sokoto more than it did the other varieties. The FS of un-infested cowpeas varied between 1 and 2 ml (Table 2). Un-infested Sokoto had the highest FS (2.0 ml) and was not affected by 25% infestation. Infestation did not affect FS of Ife brown but caused the highest reduction of 90% in that of Iron. Nevertheless, infestation decreased the mean FS of infested cowpeas by 22-37% (Table 2).

Reduction in foam properties of the infested cowpeas was similarly observed by Ojimelukwe *et al*. (1999). Kinsella (1979) reported that foam formation and stabilization is a surface-active function of proteins while Okaka and Potter (1979) observed a direct relationship between foaming properties and protein solubilities. Ojimelukwe *et al.* (1999) further noted that the rate of collapse, which is an index of foam stability, depends on the extent of protein denaturation and alterations in the protein molecules. The observed decrease in FC and FS of the infested cowpeas could therefore be attributed to decrease in protein especially in soluble protein fractions as well as to protein denaturation by infestation. Foaming capacity and foam stability properties are useful in preparing '*akara'* as the foam formed when cowpea paste is whipped holds the incorporated air and enables the bean balls to retain their unique spongy and firm shape after frying. Un-infested Sokoto white having high FC and twice FS in relation to the other varieties can be considered as having better foaming properties than the other varieties; and this suggests why it is often preferred locally in the preparation of '*akara'* bean balls. The FC of un-infested cowpeas ranged from 7.5 ml in Iron to 17.0 ml and 16.0 ml in Ife brown and Sokoto, respectively (Table 2).

Table 1: Generation of samples of infested and un-infested cowpeas

	Infestation level (%)							
	0		25		50		75	
Cowpea varieties	**Un-infested**	**infested**	**Un-infested**	**infested**	**Un-infested**	**Infested**	**Un-infested**	**Infested**
Ife-brown	1000	0	750	250	500	500	250	750
Iron	1000	0	750	250	500	500	250	750
Isiocha	1000	0	750	250	500	500	250	750
Patasco	1000	0	750	250	500	500	250	750
Sokoto	1000	0	750	250	500	500	250	750

Table 2: Effect of insect infestation on the protein content, total dietary fiber and swelling index of five cowpea varieties

Infestation %	Variety	Protein %	TDF %	BVI (ppm)	SI (ml)	FC (ml)	Fs(ml))
	Ife-brown	22.75	41	51.25	1.61	17.0	1.0
	Iron	22.75	49	37.50	1.32	7.50	1.0
0	Isiocha	15.31	49	47.50	1.49	13.0	1.0
	Patasco	21.56	34	50.00	1.29	11.0	1.0
	Sokoto	17.50	40	37.50	1.39	16.0	2.0
	Mean	**19.97 ±3.38^{a}**	**42.60 ±6.43^{a}**	**44.75 ±6.75^{c}**	**1.42 ±0.13^{a}**	**12.90 ±3.85^{a}**	**1.20 ±0.45^{a}**
	Ife-brown	17.88	32	52.50	1.55	17.0	1.0
	Iron	12.25	47	57.50	1.31	8.0	0.5
25	Isiocha	13.58	42	62.50	1.43	8.0	0.7
	Patasco	19.69	31	41.25	1.23	10.0	0.5
	Sokoto	15.25	36	55.00	1.39	13.0	2.0
	Mean	**15.73 ±3.05^{b}**	**37.60 ±6.80^{b}**	**53.75 ±7.91^{c}**	**1.38 ±0.12^{b}**	**11.20 ±3.83^{ab}**	**0.94 ±0.63^{b}**
	Ife-brown	19.94	30	80.00	1.50	13.0	1.0
	Iron	11.38	44	55.00	1.27	8.5	0.5
	Isiocha	13.22	38	65.00	1.33	7.5	1.0
50	Patasco	20.56	33	62.50	1.21	11.0	0.5
	Sokoto	10.78	33	70.00	1.25	11.0	1.5
	Mean	**15.18 ±4.72^{b}**	**35.60 ±5.50^{bc}**	**66.50 ±9.29^{b}**	**1.31 ±0.11^{c}**	**10.20 ±2.20^{b}**	**0.90 ±0.42^{b}**
	Ife-brown	18.81	29	80.00	1.36	13.00	1.0
75	Iron	21.00	47	70.00	1.24	3.50	0.1
	Isiocha	14.75	32	98.00	1.26	5.30	0.9
	Patasco	17.94	31	67.50	1.11	10.00	0.5
	Sokoto	15.89	29	87.00	1.17	6.00	1.3
	Mean	**17.68±2.46^{ab}**	**33.60±7.60^{c}**	**80.50±12.53^{a}**	**1.23±0.09^{d}**	**7.56±3.86^{c}**	**0.76±0.47^{b}**
CV%		**15.10**	**7.86**	**13.46**	**2.16**	**17.98**	**21.05**

CONCLUSION

Insect infestation adversely affected the protein content, TDF, SI, FC, FS and BVI of the cowpeas studied. A moderate infestation level of 25% adversely affected most of the properties evaluated. The severity of damage caused by the insects to the properties determined in the cowpeas was generally proportional to the infestation level, occurring most in 75% infested cowpeas. The TDF and SI of Iron was least affected by insect infestation while its protein content and foaming stability was most affected. On the other hand, patasco was generally least affected by insect infestation in protein content, level of starch damage and foaming capacity while Ife brown was the least affected in foaming stability. However, un-infested Sokoto white had better foaming properties with a high foaming capacity and foam stability twice that of the other varieties. Since with a high foaming capacity and foam stability twice that of the other varieties. Since insect infestation causes damage to both the nutritional and functional properties of cowpea, urgent measures to safeguard cowpea from infestation to help reduce food insecurity and malnutrition, especially in the developing world is needed. Efforts at improving simple technologies that can be employed at household level, especially in the use of natural insecticides (e.g. spices) to safeguard cowpea should be intensified. In addition, dissemination of information among rural women on such technologies is highly recommended.

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