



## Original Research Article

### Reduction of the Nutritional Value of Cowpea Infested with *Callosobruchus maculatus* (Coleoptera: bruchidae)

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#### ABSTRACT

This study was carried out to study the food nutrients such as the free fatty acids and some other nutritional values of cowpea infested with *Callosobruchus maculatus* in vitro. 400 g of uninfested cowpea was divided into two parts of 200 g each. The first 200 g was left to be infested by *Callosobruchus maculatus* for 100 days. The remaining 200 g was preserved in a Kilner jar in the laboratory. The mean moisture content of the infested cowpea was 21.10 while that of the uninfested cowpea was 32.70. The mean dry matter content of the infested cowpea was 78.89 while that of the uninfested cowpea was 67.30. The mean fat content of the infested sample was 2.75 while that of the uninfested sample was 6.23. The mean ash content of the infested sample was 1.85 while that of the uninfested sample was 1.90. The mean crude fibre content of the infested sample was 3.45 while the value for the uninfested sample was 2.84. The mean crude protein content values of the infested sample were 14.90 while the uninfested was 50.50. The mean carbohydrate content value of the infested sample was 21.74 while that of the uninfested sample was 5.87 and finally, the mean FFA value of the infested sample was 15.51 while that of the uninfested sample was 21.80.

#### Keywords

Cowpea,  
*Callosobruchus maculatus*,  
Free Fatty Acid,  
Infestation  
In-Vitro And  
Kilner Jar

#### Introduction

Cowpea, *Vigna unguiculata* (L.) Walp is one of the five most important legumes in the tropics and provides the protein for most people in the region and nitrogen to the soils (Duke, 1990). The cowpea seed beetle, *Callosobruchus maculatus* (Fab), is the most important storage pest of cowpea throughout the tropics (NRI, 1996). The quantity of cowpea lost annually through *C. maculatus* is substantial, although no accurate figures are available; cowpea damage estimates have always been expressed in percentages.

For instance, Caswell (1980) indicated 50% loss at Ibadan and minimally above 30% at Zaira. Umeozor (2005) also reported that *C. maculatus* damage level to cowpea reached 10% to 50% respectively. Throughout tropical Africa, *C. maculatus* consumed 50-90% of cowpea in storage annually (IITA, 1989). Frequently, farm storage for six months was accompanied by 70% seed infestation and about 30% weight loss and virtually unfit for consumption (Singh and Jackai, 1985). These percentages losses are

mere estimates. The quantification of cowpea losses through *C. maculatus* is very desirable.

Cowpea, (*Vigna unguiculata* (L.) Walp) an annual crop is the most important legume crop growth throughout the tropical belt (Singh and Rachie, 1985). Cowpea is a hardy crop but it host many pests that attack vegetables, including leafminers, whiteflies, leafhoppers, mites, thrips and aphids. Cowpea's attraction for insects may be an advantage if the planting also attract a sizable population of beneficial insects, but it is not if pest outbreaks occur and then move on to attack a cash crop. Careful weekly monitoring is important to ensure that the cowpea planting is not becoming a source of pests on the farm. The crop is particularly vulnerable to attack by lamb's tail pod rot caused by *Choanephora cucurbitarum* (Cuthebert and Ferry, 1975). Initial symptom of the disease is characterized by a water soaked areas on pod subsequently developing into a wet rot affecting both young and mature pods. Diseased pods which later turn brownish black bear luxuriant whitish growth of the casual fungus with black headed pin-like spring structures. This disease which could also be found on the stem and the flowers is favoured by high humidity and pod borer damage under the prevailing warm and humid conditions of the rain forest belt of southern Nigeria. Infection may result in total crop failure (Singh and Allen, 1979). The causal fungus causes disease on cowpea via feeding damage and oviposition puncture made by the insect *Chalcoderis aevius* (Cuthbert and Ferry, 1975) or other pest of beans such as *Maruca testulalis* (Irvine 1957, Karel and Matary, 1983). Insecticide application has been considered to be a possible method of controlling the disease (Singh and Allen, 1979).

*Callosobruchus maculatus* (Cowpea brunched), several grain legumes are attacked by this pest. However, cowpea and bambara groundnut are its main hosts. (Lale, 2002).

Economic Importance: *Callosobruchus maculatus* originated in West Africa but it has now attained a tropicopolitan distribution. It is the most important pest of stored cowpeas and bambara groundnuts in the tropics and it is responsible for a significant loss in weight and the formation of powder. Infestation of cowpeas and bambara groundnuts may commence in the field and rapid multiplication occurs in the store where conditions are ideal for its development. *C. maculatus* is, however, a storage species that is capable of commencing attack in the field.

Life History of *Callosobruchus maculatus*: the adult female lays between 70 and 100 eggs singly on the surface of the seeds. Each egg is glued firmly to the surface of the pod or seed. The optimum conditions for oviposition and further development are 30-35°C and 70-90%rh. Hatching takes place about 6 days after oviposition and each developing scarabaeiform larva spends its entire life of about 20 days within a single grain. As the larva feeds, it prepares a chamber covered with a flap of testa known as a window before pupation. The window is the hole with a thin membrane of testa covering it excavated by the larva and which serves as an exit for the eventual emergence of the adult bruchid. Pupation takes place in this chamber and the pupal period is about 7 days. On a suitable diet and under favourable conditions, adults emerge in about 3-4 weeks after oviposition. The adults are small, compact, brownish beetles 2.5- 3.5mm in length characterized by serrated and unclubbed antennae, relatively long legs, a pair of distinct and toothed

ridges on the ventral side of each hind femur and showing sexual dimorphism in the markings on the elytra. These dorsal spots are more prominent in the females which are also larger in body size than in the smaller males. Also, the last abdominal segment in both sexes is not covered by the elytra.

The infestation of cowpea by *Callosobruchus maculatus* is said to cause weight loss to the infested cowpea because most of the inner parts of the grains will have been eaten up by the pest. The weight loss according to (Umeozor, 2005) is said to alter the free fatty acid content of the infested bean. Increased plasma free fatty acid (FFA) concentrations are typically associated with many insulin-resistant states including obesity and type 2 diabetes mellitus (1-3). Furthermore, raising plasma FFA levels in healthy humans, by triglyceride/heparin infusions, can also acutely induce insulin resistance (4-11). Over thirty years ago, Randle et al (2002) demonstrated that FFAs compete with glucose for oxidation in isolated rat heart and diaphragmatic muscle preparations, and they speculated that increased fat oxidation may cause the insulin to resistance associated with diabetes and obesity. They proposed that increased FFA oxidation leads to an increase in the intramitochondrial acetyl-coenzyme A (acetyl CoA) and reduced/oxidized nicotinamide adenine dinucleotide (NADH/NAD<sup>+</sup>) ratios, resulting in inactivation of pyruvate dehydrogenase activity. The consequent increase in intracellular citrate concentration causes inhibition of phosphofructokinase resulting in an increase in glucose- 6-phosphate levels. The elevated glucose 6-phosphate levels would inhibit hexokinase II activity and then lead to decreased glucose uptake (Dresner *et al.*, 1998).

Considering these information gaps, this

project work is therefore undertaken to achieve the following objectives;

- To compare the free fatty acid value of the infested cowpea with that of the uninfested cowpea
- To determine the weight loss of the infested cowpea with that of the uninfested cowpea
- To investigate the effects of *Callosobruchus maculatus* on the nutritional value of infested cowpea

## **Material and Methods**

### **Study area**

400 g of fresh uninfested sample of oloyin (cowpea) was purchased from two major markets, Osiele market and Adatan market each in Abeokuta, Ogun State. The 400 g sample was divided into two of 200 g each. The first 200 g sample was preserved in a Kilner jar to preserve and prevent infestation by *Callosobruchus maculatus* and the remaining 200g was kept in a flask container for 100 days with excess heat which induced the breeding of *Callosobruchus maculatus* larvae.

The samples were tested to determine the moisture content, dry matter content, fat content, ash content, crude content, crude fibre content, crude protein content, carbohydrate content and the free fatty acid percentage. The weight loss of the sample was also determined before the test was carried out to know if there is difference in weight loss.

### **Proximate analysis**

#### **Moisture content**

Porcelain dishes were washed and dried in the moisture oven, put in the desiccator to

cool, and weighed. The samples were put into the dishes and weight of the dish plus the wet samples were noted. The samples were then placed in the moisture oven at 70 – 80°C for 20 hours and at 100-135°C for the next 4 hours. The samples were cooled in the desiccator and the dry weight of the sample + dish was taken.

### **Percentage dry matter**

This was obtained by deducting the moisture content from 100.

### **Ash content**

2.5 g finely grounded fine samples was accurately weighed into a porcelain crucible and charred on Bunsen flame inside a fume cupboard to drive off most of the smoke. The sample was transferred into a preheated muffle furnace at 600°C and heated at this temperature for 2 hours, after which it was transferred to a desiccators to cool. The grey or white ash obtained was then weighed.

### **Crude fibre determination**

1g of the sample was weighed into 500ml conical flask and 100ml digestion reagent was added to wash down the sides of the flask. The mixture was boiled and refluxed for 40 minutes, using a water jacket condenser to prevent loss of liquid. The flask was then removed from the heater, cooled under cold tap, filtered through a 15cm of No 4. Whatman paper, and washed 6 times with hot water and once with methylated spirit.

The Whatman paper was then opened out, and the residue removed with a spatula and the fibre transferred to a silica dish. This was dried overnight at 105°C, transferred to a dessicator and weighed when cool. It was ashed at 600°C overnight in muffle furnace, cooled in a dessicator and weighed.

### **Fat determination**

The fat content was determined using the Soxhlet method. 250cm<sup>3</sup> clean boiling flasks were dried in oven at 1100C for 30 minutes and the transferred into a desiccator and then allowed to cool. 2g of samples was accurately weighed into labeled thimbles. And also the labeled cooling boiling flasks were weighed.

The boiling flasks were filled with about 300cm<sup>3</sup> of petroleum ether and the extraction thimble was lightly plugged with cotton wool. The soxhlet apparatus was assembled and allowed to reflux for about 6 hours, thimble was then removed with care and petroleum ethers was collected in the top container of the set-up and drained into a container for re-use.

When the flask was almost free of petroleum ethers, it was removed and dried at 1050C-1100C for one hour, transferred into a dessicator, allowed to cool and weighed.

### **Acid value**

25cm<sup>3</sup> of diethylether will be added to 25cm<sup>3</sup> of alcohol and 1cm<sup>3</sup> of 1 percent phenolphthalein. 1g of each sample will be dissolved in the mixed neutral solvent and will be titrated against aqueous 0.1M NaOH solution and shaken constantly until a pink color which persists for 15 sec is obtained.

### **Free fatty acid**

This will be calculated as oleic acid. In this case,

Free fatty acid = ½ acid value  
1cm<sup>3</sup> of 0.1M NaOH = 0.0383g of oleic acid  
Methods by Association of Official Analytical Chemistry (A.O.A.C) (1998).

## Weight loss

The weight loss of the infested sample was determined by weighing the sample after infestation and subtracting from its weight before it got infested.

Weight before infestation- weight after infestation

## Results and Discussion

The 200 g of each samples of infested and uninfested cowpea was divided into 3 equal parts each to obtain different analytical results.

The mean moisture content of the infested cowpea was 21.10 while that of the uninfested cowpea was 32.70. The mean dry matter content of the infested cowpea was 78.89 while that of the uninfested cowpea was 67.30. The mean fat content of the infested sample was 2.75 while that of the uninfested sample was 6.23. The mean ash content of the infested sample was 1.85 while that of the uninfested sample was 1.90. The mean crude fibre content of the infested sample was 3.45 while the value for the uninfested sample was 2.84. The mean crude protein content values of the infested sample was 14.90 while the uninfested was 50.50. The mean carbohydrate content value of the infested sample was 21.74 while that of the uninfested sample was 5.87 and finally, the mean FFA value of the infested sample was 15.51 while that of the uninfested sample was 21.80 (Table 1).

The weight of the sample cowpea before infestation was 200g, and after infestation by *C. maculatus*, the weight was again taken to determine the weight loss and it was discovered to have reduced to 189g showing a decrease of 11g. The uninfested cowpea sample (A) which was 200g before the

analysis was carried out still remained constant at 200g. The 200g of the infested sample (B) was reduced in weight as a result of the infestation by *Callosobruchus maculatus*. Its weight reduced to 189g showing a weight loss of 11g in the weight (Table 2).

Bean beetles, *Callosobruchus maculatus* (Coleopter: Bruchidea), are tropical and subtropical agricultural pest insects that infest dried cowpeas and other related stored seeds. Damage appears as round holes in the cowpeas. They have mouthparts for chewing. They prefer dried cowpeas but will attack other beans and peas in storage. Adults move about readily and can infest seeds in the field, but can also breed continuously in stored dry cowpeas. The quantity of cowpea lost annually through *Callosobruchus maculatus* is substantial although no accurate figures are available; cowpea damage estimates have always been expressed in percentages. For instance, Caswell (1976) indicated 50% loss at Ibadan and minimally above 30% at Zaria. Throughout tropical Africa, *Callosobruchus maculatus* consumed 50 – 90% of cowpea in storage annually (IITA, 1989). All these show that *Callosobruchus maculatus* causes loss of weight of infested cowpea and decreases its nutritional value.

The infestation of cowpea by *Callosobruchus maculatus* from the analysis carried out also shows that *Callosobruchus maculatus* causes decrease in the free fatty acid value of cowpea. Free fatty acid is an acid which is very significant in the human body in moderate amount. Free fatty acid was also discovered to have effects on cellular processes, and patients with liver disease have an abnormal intermediate carbohydrate metabolism (Amatuzi, 1957), and so might theoretically use fat as a major alternative source of energy. From the

results of the analysis carried out on the infested cowpea, it shows that *Callosobrunchus maculates* also decreases the free fatty acid value of infested cowpea.

Randle *et al* (2002) demonstrated that FFAs compete with glucose for oxidation in isolated rat heart and diaphragmatic muscle preparations, and they speculated that increased fat oxidation may cause the insulin resistance associated with diabetes and obesity. They proposed that increased FFA oxidation leads to an increase in the intramitochondrial acetyl-coenzyme A (acetyl CoA) and reduced/oxidized nicotinamide adenine dinucleotide (NADH/NAD+) ratios, resulting in inactivation of pyruvate dehydrogenase activity. The consequent increase in intracellular citrate concentration causes inhibition of phosphofructokinase resulting

in an increase in glucose-6-phosphate levels. The elevated glucose-6-phosphate levels would inhibit hexokinase II activity and then lead to decreased glucose uptake. (Dresner *et al*, 1998).

*Callosobrunchus maculates* is a pest that causes great damage to cowpea and it reduces the nutritional values of the cowpea by decreasing the weight and the free fatty acid along with other variables. This study has been able to establish that the infestation of cowpea by *Callosobrunchus maculates* renders the infested cowpea nutritionally deficient and makes it unsuitable for both commercial and private consumption and private consumption. More funding should be given by government and corporate organizations for the purpose of scientific study which will help in the control of insect pests.

**Table.1** Moisture content, dry matter content, fat content, ash content, crude fibre content, crude protein content, carbohydrate content, free fatty acid percentage content of infested and uninfested cowpea samples

S/N	Sample	Moisture content	Dry matter content	Fat content	Ash content	Crude Fibre content	Crude protein content	Carbohydrate content	Free fatty acid (%)
1.	Infested 1	21.07	78.93	2.72	1.84	3.45	48.96	21.96	15.16
2.	Infested 2	21.10	78.90	2.77	1.83	3.43	48.75	22.12	15.20
3.	Infested 3	21.15	78.85	2.75	1.87	3.48	48.98	21.74	15.18
4.	Uninfested 1	32.45	67.55	6.22	1.92	2.80	50.78	5.88	21.62
5.	Uninfested 2	32.62	67.38	6.25	1.89	2.84	50.69	5.71	21.97
6.	Uninfested 3	33.02	66.98	6.21	1.90	2.77	50.02	6.08	21.82

Proximate analysis results are in g/100g sample

**Table.2** Determination of weight loss

Sample	Weight before infestation	Weight after infestation
Infested cowpea	200g	189g

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