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Crop Protection

Susceptibility of three varieties of Bambara groundnut (Vigna subterranea L.) towards Callosobruchus maculatus according to their nutritional and antinutrient contents

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The leguminous seeds of voandzou (*Vigna subterranea* L.) are an important source of protein and anti-nutrients. The present study analyses nutritional and antinutrients content of 3 varieties of voandzou during storage under attacked by Callosobruchus maculatus. Three varieties of voandzou seeds with distinct colors: white cream (CM/EN/DW/14), red (CM/EN/DW/07) and black (CM/EN/DW/28) were collected from local markets and stored in flasks for 3 months. Thereafter, these seeds were sieved and weighted per batch. Chemical analysis of nutritional potential according AOAC methods and Phytic acid, Tannin, Oxalate and the trypsin inhibition activity were conducted both on clean and infested voandzou seed samples. The results showed that the CM/EN/DW/14 seed variety was the most attacked (75.4%) followed by CM/EN/DW/07 (42.6%). Nutritional properties indicate that CM/EN/DW/14 had the highest nutrient contents, highest ash and moisture content, while CM/EN/DW/28 recorded the lowest nutritional content compared to the other two samples. Concentrations of studied anti-nutritional factors were significantly different according to the color. The black color has the highest concentration of anti-nutrients content compared to the red and the white cream color. Seeds with higher anti-nutrients content had less attack rates compared to the others.

Keywords: Voandzou, *Callosobruchus maculatus*, Antinutient contents, Storage ability

INTRODUCTION

In most African countries, subsistence agriculture is made to produce food for human consumption and raw materials for the industry (Ibrahin and Ogunwasi, 2016) using traditional methods. These current levels of production are obviously not enough to meet the growing demand for food population (FAO, 2006). Indeed, there is no closer relationship between the increase of food production that is arithmetic and the growth of the population which is exponential. The world's population was estimated to 7 billion nowadays and is expected to reach 9 billion by 2050 (Jacquet, 2010). About 795 million peoples, or 1 out of 9 peoples, are under nourished, including 90 million children under 5 years of age (FAO, 2016). Most of them (780 million peoples) live in developing countries, particularly in Africa and Asia. Sub-Saharan Africa has highest values, with nearly 25% of the population undernourished (FAO, 2016). To solve this problem, agricultural production needs to be improved to promote food security.

The leguminous seeds and singularly the voandzou represent an important source of protein. It is a plant with pods that comes in various forms: tree, shrub, grass or liana. Some are of great economic and medicinal value and most species are edible (Mabhaudhi et al., 2013). As a subsistence food crop, voandzou is likely to contribute to the food security of populations (Pumlani,

2014). It is cultivated traditionally and consumed throughout the north of Cameroon. Generally associated with cereals, the voandzou was, until recently, reserved for family self-consumption (Ngamo et al., 2016). Part of the production is now for sale in urban markets and for export to Nigeria and Chad (Omoikhje et al., 2006). Given the climate in this region of the country, marked mainly by a short rainy season (3 to 6 months on average) and a long and harsh dry season; the production of this leguminous is seasonal and its consumption extends throughout the year. The peasants store almost all of their crops in the granaries. During this storage, different factors depreciate the food, among them attacks of insect pests, where Callosobruchus maculatus are specially important (Ngamo et al, 2016). Generally grown in the same plots or those close to the cowpeas, the infestation begins in the field at the pod level and continues in granaries. These contaminations by the insects depreciates strongly the nutrient content of this staple food (Goudoum et al., 2016). Voandzou could occupy a prominent place in the economic system of this part of the country because of its transformation into ready-to-eat products (koki), which is mainly an activity reserved for women. High in protein (around 20%) and in sugars (around 50%), this legume is also a very important nutrient for local populations, who are prone to protein malnutrition problems (Yao et al., 2015).

This protein may not be readily bioavailable due to the presence of anti-nutrients. Anti-nutrients are substances that reduce the nutritional value of foods by interfering with the bioavailability of minerals and the digestibility of essential nutrients (Ames et al., 1990). These anti-nutrients are mainly present in the voandzou to different proportions according to the varieties. Trypsin inhibitors reduce the activity of digestive enzymes, while other anti-nutrients such as hemagglutinins inhibit nutrient uptake and phytates and oxalates bind to minerals that inhibit the absorption of zinc and iron (Ames et al., 1990). Lawrence and Koundal (2002) and Sharma (2015) showed that tripsin inhibitors present in the plant protect them against insect pest infestation. In the same way, the brown voandzou seeds contain more tannins than the cream colored ones (Nwokolo, 1996). These antinutrients could play an important role in the ability of these different varieties of Bambara groundnut to conservation. Thus, this research aims to compare the conservation ability of three varieties of voandzou according to their nutritional composition and their antinutrients content in the presence of C. maculatus.

MATERIAL AND METHODS

Insects rearing

A culture of C. maculatus adults used for the study was obtained from naturally infested stored Bambara groundnut seeds. The culture was maintained and reared in a ventilated incubator (Memmert Gmbh) in the laboratory (33.5 ± 4.2 °C and 57.9 ± 8.1 % relative humidity) at Maroua on the dry Bambara groundnut seeds in the rearing bottle (one L) covered with muslin cloth. Insects were placed at 25 couples in one-liter jars containing 1 kg of untreated Bambara groundnut seeds brought from Yagoua market. This phase lasted for 30 days to allow the emergence of new adults. After this operation, the Bambara groundnut seeds contained in the different jars were screened; adult weevils were removed and breeding continued with eggs infested Bambara groundnut seeds. This second phase lasted 30 days and 3 days emerged insects were used to infest Bambara groundnut seed samples of the different treatments.

Collection of Bambara groundnut seeds samples and observation

Bambara groundnut seeds samples were obtained from Yagoua marked in Mayo-Danay Division in Far-North region of Cameroon, during a prospection. Three varieties of Bambara groundnut seed samples with distinct colors: cream-colored (CM/EN/DW/07), red (CM/EN/DW/14) and black (CM/EN/DW/28) were collected from these markets. Previous studies have demonstrated that the cream-colored Bambara groundnut seeds were more attacked than the other colors (Goudoum et al., 2016). Before storage, Bambara groundnut seeds were stored for 7 days at 4 °C, to eliminate

any form of insects. Inside each batch, seed samples were infested with 20 C. maculatus of three days age (10 female and 10 males). Three repetitions of each variety are made.

The constituted pots were stored for 3 months at 33.5 ± 4.2 °C and 57.9 ± 8.1 % relative humidity. After 3 months of storage, the grains were sieved using a Xinxiang Tongxin made sieves with 3 mm diameters and weight per batch was obtained using a Kern made balance (precision 1/1000 g).

Chemical analysis of Bambara groundnut seeds before the three months storage delay

Some chemical characteristics analyzes were conducted before 3 months of storage to the infected Bambara groundnut seed samples. The seed samples were reduced to flour before analysis.

Nutritional analysis of Bambara groundnut seeds before the three months storage delay

The protein content was given according to the Kjeldahl method of the total nitrogen determination (AOAC, 1990). Lipid (fat) content was determined according to the Soxhlet method 960.39 (AOAC, 2002). Sugars were extracted and proportioned by Fischer and Stein method (1961). Ash content was determined by AOAC (2000) method. The hot air oven method, AOAC (2000) was used for moisture determination. The crude fiber content was determined using dilute acid and alkali hydrolysis (AOAC, 1995).

Anti-nutrients determination of Bambara groundnut seeds before the three months storage delay

Phytic acid was extracted from each Bambara groundnut flour sample by the method of Wheeler and Ferrel (1971). About 5 g of ground sample was placed in a 125 ml Erlen and then 25 ml of 3% trichloroacetic acid was added and the mixture stirred slightly for 45 min at room temperature. 8 ml of the mixture were removed and centrifuged at 20,000 rpm for 15 min. The phytic acid in the supernatant was precipitated as ferric phytate and iron in the sample was estimated. Phytate-phosphorus (phytate-P) was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio. The phytic acid was estimated by multiplying the amount of phytate-phosphorus by the factor 3.55, based on the empirical formula C6P6O24H18.

Tannin contents were determined by the modified vanillin-HCl methods (Price et al., 1978). A 2 g sample was extracted with 10 ml 70 % acetone for 20 min at room temperature with constant agitation. The mixture was stirred with a magnet bar for 15 minutes and then vacuum filtered through a sintered glass using a Buchner. The residue was rinsed twice with 10 ml of solvent and the acetone of the filtrate was removed with a Rotavapor at 40 °C. Correction for interference light natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction, but without vanillin reagent. The sample solution was read at 550 nm. A standard curve was prepared using catechin (Sigma-Aldrich now) after correcting for blank and tannin concentration was expressed in g/100 g. 0.2 g of (+) catechin was also treated as the sample for reference and calibration was performed using a solution of (+) catechin 5.16 mg / ml.

Oxalate was determined by AOAC (1990) method. 1 g of the sample was weighed into 100 ml conical flask. 75 ml of 3 M H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using whatman No. 1 filter paper. The precipitate retained on the filter paper was washed several times with hot water until a volume of about 60 ml. The filtrate obtained then received 3 drops of methyl red and 25% ammonia until a weak yellow coloration was obtained. The solution was then boiled in a water bath, cooled in an ice bucket and filtered to remove the precipitate of ferrous ions. The sample filtrate (extract) (25 ml) was collected and titrated against hot (80 - 90°C) 0.1 N $KMnO_4$ solution to the point when a faint pink color appeared that persisted for at least 30 s. It was then filtered and the calcium oxalate precipitate retained on the paper was transferred to a beaker with distilled water and 25% sulfuric acid until complete dissolution. To precipitate the heavy metals, 5 ml of the tungstophosphonate

reagent was added to the acidified extracts and the mixture was centrifuged at 5000 rpm for 15 min. The supernatant was subsequently heat-titrated with constant stirring with a solution of 0.05 M potassium permanganate. A control was carried out in parallel and the calibration was carried out using a solution of oxalic acid 0.2 M. The concentration of oxalate in each sample was obtained from the calculation: 1 ml 0.1 permanganate = 0.006303 g oxalate.

The trypsin inhibition activity was assayed in terms of the extent to which an extract of the defatted flour inhibited the action of bovine trypsin (EC 3.4.21.4) on the substrate benzoyl-DL-arginine-p-nitrianilide (BAPNA) hydrochloric (Kakade et al., 1974). About 1 g of ground sample was extracted with 10 ml of 0.1 M NaCl solution (prepared with borate buffer pH 8.5) for 5 hours of stirring. At the end of the extraction period, the mixture was centrifuged at 1500 rpm for 20 min. The pellet was again extracted with 10 ml of the NaCl solution for 20 min and then centrifuged at 1500 rpm for 20 min. Both supernatants were mixed and used for trypsin inhibitor analysis. The pH of the resulting slurry was adjusted to 9.4 - 9.6 with 1 M NaOH. After extraction, the suspend ion was shaken and diluted with distilled water such that 1 cm3 of the extract produced trypsin inhibition of 40 - 60% at 37°C. The respective dilutions were noted. Consequently, TIA was calculated in terms of mg pure trypsin (Sigma-Aldrich now):

Where D is the dilution factor, A is the change in absorbance at 410 mm due to trypsin inhibition per cm3 diluted sample extract and S is the weight of the sample.

Statistical analysis

The results obtained from the evaluation of nutritional and antinutritional properties of Bambara groundnut grains during 3 months storage were analyzed with analysis of variance (ANOVA) using the software Xlstat 2018. The average values were classified using Duncan Multiple Test with the same software.

RESULTS

Analysis of the attack rate of the three Bambara groundnut seed samples

Table 1 shows the attack rate of three Bambara groundnut seed samples by C. maculatus after three months storage.

It appears from the table 1 that unprotected and infected Bambara groundnut seeds are attacked by pests insects with a significant rate ($p < 0.01$) between different varieties. CM/EN/DW/14 seed variety is the most attacked (75.4%) followed by seed sample of CM/EN/DW/07 (42.6%) while the lowest attacked seeds was recorded in the CM/EN/DW/28 sample (20.8%). Regarding the colors, it appears from this study that Bambara groundnuts having a cream-color are the most damaged by C. maculatus attack.

Nutritional composition of the three Bambara groundnut seed samples

The compositional analysis (% dry weight) studied of the three varieties of Bambara groundnut seed samples are shown in the table 2.

The results showed that moisture, ash, crude lipid, crude fibre, crude protein and crude carbohydrate varied with the varieties of Bambara groundnut seeds.

The moisture content of the seeds was highest in the seed sample of CM/EN/DW/14 (12.1%) variety followed by seed sample of CM/EN/DW/07 (10.5%) while seed sample CM/EN/DW/28 (8.85%) recorded the lowest moisture content. There was significant difference ($p< 0.0001$) among the moisture content of the three seed samples used.

The ash content of the seeds was highest in the seed sample of CM/EN/DW/14 (5.06 %) followed by seed sample of CM/EN/DW/07 (4.08 %) while seed sample of CM/EN/DW/28 (3.63 %) recorded the lowest ash content. There was significant difference ($p< 0.0001$) among the ash content of the three seed samples used. The crude protein content of the seeds increased significantly ($p< 0.005$) from 20.3 % for the CM/EN/DW/07 sample, to 20.6 % for the CM/EN/DW/28 sample and at 23.3 % for the CM/EN/DW/14. There was no significant difference between the two last Bambara groundnut seed samples (CM/EN/DW/07 and CM/EN/DW/28) regarding crude protein content, but there was significant difference ($p< 0.0001$) between the first seed sample of (CM/EN/DW/14) and the these two last sample. The crude lipid content of the seed in the tree varieties of Bambara groundnut used was highest in a sample of CM/EN/DW/14 (8.77 %) closely followed by seed sample of CM/EN/DW/07 (7.38) and seed sample of CM/EN/DW/28 (6.83 %). There was no significant difference between crude lipid content of the last two Bambara groundnut seed samples of (CM/EN/DW/07 and CM/EN/DW/28), but there was significant difference ($p< 0.0001$) between these last two Bambara groundnut samples and the first (CM/EN/DW/14). The carbohydrate content of the seed in the three varieties of Bambara groundnut used was highest in a sample of CM/EN/DW/14 (62.2 %) followed by seed sample of CM/EN/DW/07 (58.8%) while seed sample of CM/EN/DW/28 (55.6 %) recorded the lowest carbohydrate content. There was significant difference ($p< 0.006$) among the carbohydrate content of the three seed samples used. There was no significant difference between the crude fibre content of sample CM/EN/DW/28 (4.76 %) and sample CM/EN/DW/07 (4.68%) while they are significantly different ($p< 0.005$) with the sample CM/EN/DW/14 (5.84 %).

Antinutritritional content of the tree Bambara groundnut seed samples

Antinutritional content of Bambara groundnut seed samples is presented in table 3.

The oxalate concentration in Bambara groundnut seed samples varies significantly ($p< 0.0001$) with varieties. The oxalate content of seeds was highest in the sample CM/EN/DW/28 (3.07 ± 1.04 mg/100 g) followed by seed sample CM/EN/DW/07 (2.81 ± 0.97 mg/100 g) while the lowest oxalate of seed was recorded in the sample CM/EN/DW/14 (2.23 ± 0.03 mg/100 g); respectively for the black, red and the cream-colored color seed samples. There was no significant difference between the tree Bambara groundnut seed samples regarding the tannin content. This tannin content increased between 0.80 ± 0.01 mg/100 g for CM/EN/DW/14, 1,07 ± 0.01 for CM/EN/DW/07 to 1.34 ± 0.38 mg/10 g for CM/EN/DW/28. Seed sample of CM/EN/DW/14 showed the lowest phytate concentration (36.4 ± 0.17 mg/100 g) followed by CM/EN/DW/07 (42.5 ± 0.17) while the highest phytate concentration was recorded in seed sample of CM/EN/DW/28 (46.6 ± 2.47 mg/100 g). There were significant difference ($p< 0.0001$) among the phytate content of the tree seed samples used. The trypsin inhibitor content of the seed samples decreased from 7.73 to 6.77 ± 0.01, and to 5.82 ± 0.02 mg/100 g for CM/EN/DW/07, CM/EN/DW/28 and CM/EN/DW/14 respectively. There were significant difference ($p< 0.0001$) among the trypsin inhibitor content of the three seed samples used. The concentrations of studied anti-nutritional factors were different significantly ($p< 0.0001$) with the color. The black color has the highest concentration of anti-nutritients content compared to the red and the cream-colored color, except for the trypsin inhibitor content which is higher in the red color Bambara groundnut seeds.

Figure 1 represented the level of correlation between the three seed samples by the pie chart of the principal component analysis, made it possible to visualize the groups of interacting variables.

A group of strongly and positively correlated parameters has been highlighted concerning the nutritional parameters and the attack rate. In contrast, on the first component, negative correlations with antinutrient and attack rate variables, except tanins that are strongly and positively correlated with the attack rate. This result shows that seeds with good nutritional quality are strongly attacked compared to others. The anti-nutrients factors are negatively correlated with attack rates, proves it too. When antinutrients factors are higher, nutritional parameters are lower and consequently, the attack rate is low. The correlation analysis showed that the most attacked

variety is the one with a high nutrient content and a low concentration of antinutrients (Table 4).

Proteins are strongly correlated with sugars (0.998), crude fiber (0.948) and crude lipids (0.917). These nutritional parameters are all negatively correlated with antinutritional factors. Nutritional parameters are also strongly correlated with the grain attack rate. This attack rate is negatively and strongly correlated with antinitrition factors such as tannins and oxalates. Although the correlation between water content and nutritional factors of the three grains of voandzou is significant, these factors influence the attack rate.

DISCUSSION

In view of these results, C. maculatus attacks Bambara groundnut seeds regardless of the color. However, the attack rate varies significantly ($P < 0.001$) from one color to another. These attack rates vary between 20.8% and 75.4% during 3 months storage. It can be deduced that C. maculatus could have a color preference of Bambara groundnut seeds or Bambara groundnut seeds with a dark color could not be appetizing for these insect pests.

Nutritional properties indicate that seed sample CM/EN/DW/14 was the highest nutrients, and highest ash and moisture contents, while seed sample CM/EN/DW/28 recorded the lowest nutritional content compared to the other two seed samples. So the concentration of nutrients was higher in the cream-colored seed sample (CM/EN/DW/14) followed by the red seed (CM/EN/DW/07) and black seed sample (CM/EN/DW/28) successively.

The nutritional composition of voandzou seeds shows that it is an interesting food for human nutrition. This healthy food would be of invaluable energy value. So, the results of this study showed that moisture of seed sample CM/EN/DW/14 as higher than that reported in the literature (less than 10 %) (Yao et al., 2015). Damage observed on the storage Bambara groundnut may due to this higher moisture. This moisture content in the seed sample CM/EN/DW/14 can promote attacks by insects and microorganisms.

The result showed that protein, lipid, starch and carbohydrate varied with Bambara seed varieties. The protein content also decreased significantly ($p < 0.0001$) during storage of Bambara groundnut. These Bambara groundnut compound leads to chemical degradation when insects attacks. These reactions can be accelerated, slowed or even blocked and consume energy. Thus, proteins can be degraded into their basic constitutive elements that are amino acids by the action of enzymes (proteases) in the presence of insects. They can also cause the degradation of lipids into free fatty acids which, if they are unsaturated, will be able to oxidize in the presence of air. By their metabolic activity, these insects degrade carbohydrates by producing heat and water (FAO, 2016). The heat and water vapor produced by the metabolic activity of insects tend to create a favorable environment for the development of micro-organisms. These insects can cause very serious damage by consuming the albumen and sometimes the grain germ, by depreciating the products by their waste, droppings or secretions (FAO, 2016). The ash content of Bambara groundnut seed samples range to 3.63 from 5.06 %. These proportion of ash content shown a higher mineral contents present in the food materials (Nnamani et al., 2009). These foods with high nutritional value would be subject to pest attack.

The antinutrients are located in the testa of many dicotyledonous seeds such as legumes and nuts. It is found closely associated with proteins (Khokhar and Apenten, 1986). These antinutrient factors in foods are mainly responsible for negatively impacting the absorption of nutrients in the digestive system (Unigwe et al., 2017). This antinutrients varied with the varieties and localization of Bambara seeds. Unigwe et al. (2017) reported wide variability of this antinutrients content in Bambara groundnut seeds. These authors reported a mean value of tannin ranging from 0.20 to 6.20 mg/g which are higher than those of our Bambara seed samples. Unigwe et al. (2017) reported that this variation of tannin content among different studies may be due to environmental factors or

extracting methods.

These antinutrients can also interfere with the digestive system of insects, thus preventing depredation. Tannins reduce the retention of the nitrogen fraction of the diet in the monogastric diet, resulting in a reduction in growth rate and feed efficiency (Gemede and Ratta, 2014). Tannins can also combine with proteins by reaction with lysine or methionine residues rendering them unavailable during digestion (Davis, 1981). They also reduce the palatability of food (Roeder, 1995) and are responsible for the bitterness and astringency of many foods (Bravo, 1998). Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption (Felix and Mello, 2000). If tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose and intestinal digestion may be depressed (Aletor, 2005).

Phytates form complexes with proteins either directly by establishing ionic bonds, or indirectly via a cation such as calcium, with negatively charged sides (Ravindran et al., 2010). Phytate-protein, phytate-mineral-protein complexes inhibit the digestion and bioavailability of proteins (Harland and Morris, 1995). Phytates can also interact with starch, either directly through hydrogen bond formation with a phosphate group, or indirectly via proteins resulting in decreased solubility and digestibility of starch (Harland and Morris, 1995). There was a highly significant ($p< 0.01$) difference observed for the phytic acid contents among all the accessions evaluated in this study.

These antinutrients are proteins capable of reducing the efficiency and availability of a nutrient at its site of cell use, could play an important role in the nutrition of insect pests and limit their predatory action. Plant proteinase inhibitors (PIs) have been well established to play a potent defensive role against predators and pathogens (Sharma, 2015). They also inhibit digestive enzymes and may also precipitate proteins, theses chemical compounds protected plants from insects or other animals that might feed on them (Chukwuebuka and Chinenye, 2015).

Breeding to reduce anti-nutritional factors in bambara groundnut will therefore require careful selection of genotypes. The anti-nutrients in bambara groundnut indicate that they could negatively affect the bioavailability of some vital minerals in the digestive system. These findings are in agreement with earlier work by Samuel et al. (2014) who suggested that some of the anti-nutrients in bambara groundnut negatively affect the availability of the nutrients and minerals; however, the effects were reduced through adequate processing.

CONCLUSION

The study on conservation ability of 3 varieties of Bambara groundnut seeds in storage for 3 months in presence of C. maculatus, shwed that the white cream-color sample are the most attacked than the red and black colors. The white cream color variety had the highest nutritional value and the lower antinutrients content. The black color variety showed the best conservation ability than the cream and red color. The antinutient factors can play a important role in the Bambara groundnut conservation.

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