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The Impact of Seed-Borne Fungi Associated with Roasted Cashew (*Anacardium Occidentale* L) Nuts on Its Food Value, Mineral and Anti-Nutrient Contents and Human Health

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Abstract

Isolation and identification of seed-borne fungi associated with cashew nuts from source of production and market was carried out. The proximate, mineral and anti-nutrient composition of the source of production and the market samples of roasted cashew nuts were also investigated. The result of isolation of fungi associated with the nuts from source of production and the market showed that *Claviceps purpurea*, *Aspergillus fischeri*, *Aspergillus carbonarius*, *Aspergillus flavus*, *Aspergillus tamrii* and *Aspergillus fumigatus* were associated with seeds from samples from the two sources. The ash, lipid, carbohydrate and protein contents of the source sample were relatively higher, while the moisture, fibre and vitamin C content of the markets samples were relatively lower. The Sodium, Zinc, Nitrate, Nitrogen and phosphorus contents of the market samples were observed to be higher when compared with the source sample, while the phosphate, Magnesium, Calcium, Manganese and Copper contents of the source samples were higher than the market sample. The saponin and the cyanogenic glycoside content of both market and source samples was the same, while the alkaloid content of the source samples were relatively higher. The flavonoid content of the market sample was observed to be higher when compared with the source samples.

Introduction

Cashew (*Anacardium* occidentale L) is next to cocoa as an export crop and a major source of cash income to many small holder farmers in the Central and Northern parts of Nigeria [1,2]. The Cashew Nut Liquid (CNL), a by-product of processing cashew, is mostly composed of anacardic acids [3]. These acids have been used effectively against tooth abscesses due to their lethality to a wide range of Gram-positive bacteria [4]. The bark is scraped and soaked overnight or boiled and used as an anti-diarrhea, and the seeds when ground into powders are used as anti-venom for snake bites [4]. The nut oil is used topically as an anti-fungal agent, and for healing cracked heels [4]. Anacardic acid is used in the chemical industry for the production of cardanol, which is used for resins, coatings, and frictional materials [3].

The cashew nut is a popular snack often eaten on its own. Not only do cashews have a lower fat content than most other nuts, but approximately 75% of their fat is unsaturated fatty acids, which includes oleic acid that promotes good cardiovascular health [5]. According to [6], when it is added to a low-fat diet, it can help to reduce high triglyceride levels associated with an increased risk for heart disease, and even in diabetic patients, as triglycerides are a form in which fats are carried in the blood. It is also reported that high triglyceride levels are associated with an increased risk for heart disease. Kelly and Sabate (2006), reported that consuming nuts at least 4 times a week resulted in a 37% reduced risk of coronary heart disease compared to those who never or seldom ate nuts, and that each additional serving of nuts per week was associated with an average 8.3% reduced risk of coronary heart disease [7].

According to Ensminger and Ensminger (1986), topping your morning cereal with a quarter-cup of cashews will supply you with 38.0% of the daily value for copper, known for its antioxidant effects, energy production etc [8]. Calcium is necessary for strong bones, and Magnesium is also vital for healthy bones as about two-thirds of the Magnesium in the human body is found in our bone. They give bones their physical structure, while the rest is found on the surface of the bone where it is stored for the body to draw upon [9].

People who eat nuts at least twice a week are much less likely to gain weight than those who almost never eat nuts [10]. He also reported that Zinc content in nuts plays a crucial role in blood formation, and that Copper and Zinc present even in trace concentrations are important

for physiological function of living tissues, and regulate many biochemical processes.

The anti-nutritional factors in the *A. occidentale* are Cynic Acid, Oxalate Acid, Phytatic Acid and Taninic Acid, and a daily intake of 450mg of Oxalic Acid interfered with metabolism [11]. High Oxalate levels in food may reduce the bio-availability of Calcium, and Phytic Acid intake of 4.00-9.00 mg/l00g reduces iron absorption by 4-5 fold in humans [11,12], reported that Oxalate forms insoluble complexes with Calcium, Magnesium, Zinc and Iron, interfering with the utilization of these mineral elements. The same was applicable to Phytate and Tannins with very low concentration in varieties of *A. occidentale*, though Tannins and Phytates are also know to affect human nutrition and metabolism, as high intake of tannic acid has been associated with carcinogenic effect in humans, poor protein utilization liver and kidney toxicity [11]. He further stated that Tannic Acid is associated with lower nutritive value of protein in foods, however, the concentration in these varieties did not exceed lethal levels; hence, the consumption of the nut will not pose any harmful effect to health. Phytic Acid intake of 4-9 mg/100g is thought to decrease Iron absorption by 4-5 folds in human [12].

The most predominantly encountered species in decreasing order of isolation from the non-disinfected nuts were *Aspergillus nigar*, *A. restrictus*, *A. Flavus*, *A. Fumigatus*, *Rhizopus nigrians*, *R. arrhizus* and *Macrophomina pusillus*, while *A. tamari*, *Penicillium citrinum*, *A. ochraceus*, *Penicillium Sp*; *P. digitatum* and *Syncephalastrum sp* were the less frequent isolates and were not recovered from the surface – disinfected nuts [13].

Potassium (K) is mainly associated with fungal membrane function, maintaining electrical potential across membranes, anion, cation and osmotic balance; Magnesium (Mg) is a cofactor for many enzymes; Iron contributes its own role in the oxygen carrying and electron carrying in the course of respiration; Zinc help in anaerobic respiration of fungi and can be seen in alcoholic fermentation, while Copper plays a crucial role in electron transport [14].

Some research has been done on the proximate and phyto-chemical composition of cashew nuts, as well as fungi associated with them but no information exists on the effect of fungi on the values of these proximate and phyto-chemical contents. Hence, this work is aimed at the investigating the effect of seed-borne fungi associated with roasted cashew nuts heavily consumed in the eastern part of Nigeria, and the impact on human health.

Materials and Methods

The cashew samples were collected both from the source of production where they were roasted at Nsukka Enugu State and from the market. All organic solvents and chemicals used in the analysis were obtained from the chemistry laboratory in the University of Port Harcourt, Nigeria. The reagents used were of analytical grade (BDH).

Isolation and Identification of Fungi

Cashew nuts were placed into 1% sodium hypochlorite to remove the surface saprophytes. Five seeds were placed in potato dextrose agar in Petri-dishes after they had gelled. They were incubation for 48hrs at a temperature of 25±2 °C in an incubator. Pure cultures were made of fungal species that grew on the seeds, after the 48hrs period. This was done for seeds obtained from the source of production and from the market. Ten plates were plated per sample. Identification of the organism were done using the habit character, or spore characteristics for those whose identification were not possible through the habit character [15,16].

Proximate and Phyto-Chemical Analyses of the Nuts Form the Source of Production and the Market

The proximate and phyto-chemical analyses were done [17]. The samples used were those from the source of production of the cashew nuts and from the market.

Proximate analysis

This involves Protein, Carbohydrate, Moisture, Fibre, Ash and Lipid.

Determination of protein: 1g sample was weighed and added into a clean conical flask of 250 ml capacity. 3g digestion catalyst was added into the flask and 20 ml concentrated sulphuric acid was also added and the flask was heated to digest the content from black to sky-blue colouration. The digest was cooled to room temperature and was diluted to 100 ml with distilled water.

Distillation: 20 ml diluted digest was measured into a distillation flask and the flask was held in place on an electro-thermal heater hot plate to distillation flask and attached to a Liebig condenser connected to a receive adaptor containing 10 ml 2% boric acid indictor. 40% sodium hydroxide was injected into the digest via a syringe attach to the mono-arm steel heated until the digested became strongly alkaline. The mixture was heated to boil and distill the ammonia gas via the condenser into the receiver beaker. The colour of the boric acid changed from purple to greenish as ammonium distillate was introduced into the bone acid.

Titration: The distillate was titrated with 0.1N hydrochloric acid back to purple from greenish. The volume of Hydrochloric acid added to effect change was recorded as titre value.

Calculation

$$= \%N_2 = \frac{titre\,value \times 1.4100 \times 100}{100mg \times 0.1 \times 20}$$

% protein = % N_2 x 6.25

Determination of carbohydrate: 0.1g sample was weighed into 25 ml volumetric flask, and 1.3 ml 62% perchloric acid was added and shaken for a period of 20 minutes to homogenize completely. The flask was made up to 25 ml mark with distilled water and stopped. The solution formed was filtered through a glass filter paper or allowed to sediment and decanted. 1 ml of the filtrate was collected and transferred into a 10 ml volumetric flask and diluted to desired volume with distilled water. 1 ml of the working solution was pipetted into a clean test tube and 5 ml Anthrone reagent was added. 1 ml distilled water and reagent was added and mixed. The whole mixture was read at 630nm wavelength using distilled water as blank. A standard glucose of 0.1 mg/ml was also prepared and treated as the sample with Anthrone reagent. Absorbent of the standard glucose was read and the value of carbohydrate is glucose was calculated using the formula:

$$\%CHO\,as\,glu\cos e = \frac{25 \times Absorbent\,of\,sample}{absorbent\,of\,s\tan dard\,glu\cos e \times 1g}$$

Determination of moisture: 1g sample was weighed into a clean dried porcelain evaporating dish. This heated in oven set at 105 °C

for 6 hrs. The evaporating dish was cooled in a desiccator to room temperature, and then this was re-weighted and recorded. Weight of moisture was calculated by subtracting the weight of dried sample from the fresh.

$$\%\,moisture = fresh\,weight - \frac{Dried\,weight}{Weight\,of\ fresh\,sample} \times \frac{100}{1}$$

Determination of lipid: 2g sample was inserted into a filter paper and placed into a Soxhlet extractor. The extractor was placed into a pre-weighted dried distillation flask. The solvent (Acetone) was introduced into the distilled flask via the condenser end attached to the Soxhlet extractor. The set up was held in place with retort stand and clamp. Cold water was allowed to flow into the condenser and the heated solvent was refluxed as a result. When the lipid was observably extracted completely from the sample under test, the condenser and the extractor were disconnected and the solvent was evaporated to concentrate the lipid. The flask was then dried in the air oven to constant weight and re-weighed to obtain the weight of lipid.

$$\%\,lipid = \frac{weight\,of\ fresh\,and extract - weight\,of\ flask}{Weight\,of\ sample\,extracted} \times \frac{100}{1}$$

Determination of ash: 1g sample was weighted into a Porcelein crucible which was previously pre-heated and weighed. The crucible was inserted into a muffle furnace and regulated to a temperature of 630 °C. This was heated for 3hrs and allowed to cool to room temperature and re-weighed.

$$\%\,Ash = \frac{weight\,of\ crucible + Ash\,weight\,of\ empty\,crucible}{Weight\,of\ sample} \times \frac{100}{1}$$

Phytochemical analysis

Determination of alkaloid: Using Harborne method, 5g of the sample was weighted into 250 ml beaker and 200 ml of 10% Acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was then dried and weighed.

Calculation

$$\frac{Weight\,of\ flask\,and\,sample\,residue - weight\,empty\ flask}{Weight\,of\ sample\,used} \times \frac{100}{1}$$

Determination of flavonoid: Using Harborne method, 10g of the cashew nut sample was extracted repeatedly with 100 ml of 80% aqueous methonal at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Calculation

$$\frac{Weight\,of\ flask\,and\,sample\,residue - weight\,empty\ flask}{Weight\,of\ sample\,used} \times \frac{100}{1}$$

Determination of saponins: Frothing test was used. 2 ml of the extract in the test tube was vigorously shaken with water for some time; persistent frothing showed the presence of Saponin A. 2: 500mg of the extract was placed on a freshly prepared blood agar plate and left for 6 hours. Complete haemolysis of the blood around the extract indicated presence of saponin B (Saponin glycosides).

Determination of cyanogenic glycoside: 10g of the sample was weighed to pass No 20 sieve in 800 ml Kjedahl flask. 200 ml of water was added and allowed to stand for 2-4 h; then the mixture was steam distillated. 150 ml of the distillate was collected into NaOH solution and diluted to a 250 ml with distilled water and this were titrated with 0.2N $AgNO_3$, using micro-burette. The end point was faint, but permanent turbidity and easily recognized, especially, against black background. 1m1 0.02N $AgNO_3$ = 1.08mg HCN (Ag equiv to 2CN).

Determination of tannins: Using Folin Dennis method, 0.1g sample was weighed into a clean 250 ml conical flask. 100 ml water was added and boiled for 1hr. The sample was filtered and made up to 500 ml with distilled water. 1 ml of this solution was pipetted into a clean test tube and 2.5 ml Folin Dennis solution was added, 1 ml 7% of sodium carbonate. This was allowed to stand for 20 minutes for proper colour development. A standard tannic acid was prepared and treated as to the sample. The absorbance of the colours was read at 630 nm wavelength using a blank.

The concentration of the tannin in sample was extrapolated from standard tannic acid graph.

Determination of Vitamin C: Using Indophenol method, 0.1g sample was extracted with 10% Acetic acid (glacial) l0 ml of this solution was titrated with 0.01% indophenol to a permanent pink colouration. 0.1g ascorbic acid was also dissolved in l00 ml with l0 ml was titrated with 2,6-dichioropenol indophenol to a permanent pink. The values were recorded and used to calculate the concentration of vitamin C in the sample.

Calculation

Standard vitamin C = 0.01

Volume of vitamin C = l00 ml

Aliquot titrated = 25 ml

Titrated value = 53.0 ml

25 Vitamin C = 53.0 ml indophenol

100 ml V.C = $\frac{53.0}{25} \times \frac{100}{1}$

= 212 ml indophenol

But in l00 ml V.C = 0.0lg V. C

0. 01g. V. C = 212 indophenol

∴ 212 indophenol = 0.0lg V. C

$0.5ml = \frac{0.01g}{212} \times \frac{0.5}{1} = 2.35841 \times 10^{-5}$

2. 35849 x 10 = 0.1g sample

$1000g = \frac{2.38849}{0.1} \times \frac{10100}{1}$

= 0. 235849 g/kg

None infected = 235. 85 mg /kg

Determination of nitrogen: Using Kjeldahl method, the sample was weighed into a clean conical flask. 3g digestion catalyst was added into the flask and 20 ml concentrated sulphuric acid was also added

and the flask heated to digest the content from black to sky blue colouration. The digest was cooled to room temperature and diluted to 100 ml with distilled water.

Distillation: Under distillation, 20 ml distilled digest was measured into distillation flask and the flask was held in place on the electric thermal heater hot plate, the distillation flask was attached to Leibig condenser connected to receiver adapter containing l0 ml 20% boric acid indicator. 40% sodium hydroxide was injected into the digest via syringe attached to the mono-arm steel head until the digest became strongly alkaline. The mixture was heated to boil and distilled ammonia gas via the condenser into receiver beaker. The colour of boric acid changed from purple back to greenish as ammonia distillate was introduced into boric acid.

Titration: The distillate was titrated with standard 0.1 N hydrochloric acid solution back to purple from greenish. The volume of hydrochloric acid added to effect this change was recorded as titre value.

$$\%N = \frac{Titre\,value \times 1.4 \times 100 \times 100}{100mg \times 20.0 \times 1g}$$

Titre value = the volume of HCL used in titration

1.4 = N_2 equivalent to normality of HCL used in titration.

100 = total volume the digest was made up to

100 = % factor

1000 = conversion factor from g to mg

20 = aliquot volume of digest

1g = weight of soil digested

Determination of nitrate: Using Brucine method, 1g sample was extracted with 50 ml 2.5% Acetic acid. This was filtered into clean beaker and 1 ml was pipetted into test tube with 0.5m1 Brucine reagent added. 2mls concentrated sulphuric acid was added to develop a yellowish colour in the presence of NO_3- ion. The colour formed was absorbed at 400nm using water as blank. Standard nitrate was prepared by dissolving 0.72 16g potassium nitrate in l00ml distilled water and diluted 10 times to obtain a working standard of 0.1 mg NO_3- per ml.

Determination of phosphorus: 1g of the sample was extracted with 50 ml 2.5% glacial acetic acid. The extract was filtered into comical flask and 8. 0 ml of combined reagent was added into the flask. A blank and standard phosphate ion concentration ranging from 0.0001 to 0.0007 were prepared and 0.8 ml combined reagent was added respectively. The bluish colour developed within 30 min interval, and was read at 840 nm wavelength in thermo-spectrophotometer. The sample extract volume developed was also read at the same wavelength. The concentration of the phosphate ion in the sample was extrapolated from the standard phosphate graph plotted value in the table displayed.

Atomic Absorption Spectrophotometer Method was used for the Analysis of Metals

The digests were aspirated into Atomic Absorption Spectrophotometer burner, after which it was calibrated using standard solution of respective metal to be tested. The hollow cathode lamp was given adequate time to stabilize and other settings done according to the operational manual of the Atomic Absorption spectrophotometer.

About 1g sample was digested with combined acids (Perchloric Acid 5 ml and 10 ml of Nitric Acid). The digest was diluted to 50 ml with distilled water and filtered. The filtrate was analyzed for various metal ions by atomic absorption spectrophotometer.

Sodium

589 nm wavelengths were selected. Air and gas pressure was adjusted, slit width and other settings as recommended for equipment employed were also adjusted. The instrument was calibrated with standard sodium ion concentration to obtain a standard ion graph. The samples was aspirated into the instrument and its concentration obtained by extrapolation from the standard graph in mg / L or ppm.

Potassium

766 nm wavelengths were selected. Slit width, air and gas pressure were adjusted. Other vital setting as recommended for instrument employed were programmed standard potassium ion concentration were aspirated into the instrument burner chamber to calibrate the equipment and to plot graph of the standard ion.

Magnesium

285.2 nm wavelengths were selected in the instrument. Air and gas pressure flow was adjusted. Slit and other setting as recommended for instrument lamp was also adjusted. Hollow cathode lamp was also energized by allowing adequate time, standard magnesium ion concentration were aspirated into the instrument as to effect calibration and plotting of standard graph. The sample solution was aspirated into the instrument and concentration of test ion was extrapolated from the standard graph in mg/L.

Calcium

422.7 nm wavelengths were selected. Air and gas pressure were regulated, slit width and other setting as recommended for the instrument employed were adjusted. Hollow cathode lamp was given adequate time to stabilized. Standard concentration of calcium ion was aspirated into the equipment for calibration and for the plot of standard graph. The sample was aspirated within the same condition as the standard into the equipment and result was obtained from the equipment screen.

Iron

248.3 nm wavelengths were selected. Air and gas flow were adjusted. Slit width and other vital setting as was recommended for the instrument were adjusted. Hollow cathode lamp was stabilized to allow adequate time to energize it. The instrument was then calibrated with standard Fe ion concentration to obtain a standard plot. The sample was aspirated into the instrument and concentration of Fe ion in sample was obtained by extrapolation from standard Fe ion graph in ppm or mg/L.

Manganese

279.5 nm wavelengths were selected. Air and gas pressure was adjusted, slit width and other vital settings recommended for the instrument employed were programmed and regulated. The equipment was calibrated by aspirating standard manganese ion concentrations. Standard graph of manganese was plotted, and

then sample solution was aspirated into the AAS chamber. The concentration of Mn ion in the sample was determined from the standard Mn ion graph in mg/L.

Zinc

213.8 nm wavelengths were selected. Air and gas pressure flows were adjusted. Slit width and other setting as was recommended were adjusted. Hollow cathode lamp was allowed to stabilize. The standard zinc concentrations was aspirated to calibrate the equipment and to obtain standard graph of zinc ion. Aspirator system was flushed with de – ionized water occasionally before sample solution was aspirated and the concentration of zinc ion in the sample was extrapolated and recorded in mg/L or ppm.

Copper

324.8 nm wavelengths were selected. Air and gas pressure was adjusted. Slit width and other setting as recommended for the instrument was adjusted. Hollow cathode lamp was energized and adequately allows stabilizing copper ion concentration and graph of same were plotted. Then the sample solution was aspirated into equipment and the concentration in the same was extrapolated from the standard copper graph in ppm or mg/L.

Lead

Sample was converted to ash in a Muffle furnace at a temperature of 630 °C for 3 hours. The ash was dissolved in 10ml of concentrated hydrochloric acid and was heated on an electro-thermal heater hot plate. The solution of the ash was diluted to 50 ml with distilled water. The solution was analyzed for metal ion by AA; hence, Lead ion was analyzed by AAS at 283.3 nm wavelength. The wavelength selected with a narrow slit width, air and acetylene gas flow was adjusted and other setting as recommended for the instrument employed was attended to and regulated. Hollow lamp cathode was given adequate time to stabilize before aspirating standards solution for equipment calibration. After calibrating the equipment with standard lead concentration, the system was flushed with distilled water severally before aspirating the sample's solution on the sample experimental condition used for the standard. The concentration of lead ion in the sample was extrapolated from the standard graph of lead ion plotted.

Result

The result of isolation of fungi associated with the nuts from source of production and the market showed that *Claviceps purpurea*, *Aspergillus fischeri*, *Aspergillus carbonarius*, *Aspergillus flavus*, *Aspergillus tamrii* and *Aspergillus fumigatus* were associated with seeds from samples from the market and source of production. Results in table 1 shows the proximate and the Vitamin C composition of the source and market samples of the cashew nut. It was observed that there was a decrease in the ash, lipid, carbohydrates and protein contents of the market sample of the cashew nuts, while there was a relative increase in the moisture, fibre and the vitamin c content of the market samples. A correlative test carried out showed that there was no significant different ($p>0.05$) in the results.

As shown in Table 2 the metallic mineral content of the source of production sample of the cashew nut and that of the market show that the Sodium (Na) and Zinc (Zn) contents of the market sample were relatively Copper (Cu), contents of the sample were relatively higher. The Cadmium (Cd) and Lead (Pb) contents of the samples were however very negligible. The non-metallic minerals content of the source of production sample and the market showed that the Nitrate, Nitrogen and phosphorus contents of the market sample were higher

Table 1: Proximate and vitamin c composition of the production source sample of the cashew nut and the market sample.

Metallic mineral	Production Source sample conc. (%) Mean ± SD	Market sample conc. (%) Mean ± SD
Ash	2.92 ± 0.04	2.13 ± 0.05
Lipid	49.21 ± 0.01	45.24 ± 0.04
CHO	12.64 ± 0.08	8.48 ± 0.09
Protein	21.85 ± 0.031	17.52 ± 0.03
Moisture	1.68 ± 0.003	4.91 ± 0.04
Fiber	11.70 ± 0.14	21.65 ± 0.08
Vitamin	47.16 ± 0.02	47.17 ± 0.01

($p>0.05$)

when compared with those of the source of production sample, while the phosphate content of the source of production sample were relatively higher. A correlation test carried out, however, showed that there was no significant difference between the results from the samples from the source of production and that from the market ($p>0.05$). As shown also in Table 3, the alkaloids content of the source of production sample was higher than those from the source, while the flavonoid content of the market sample was relatively higher. The saponins and the cyanogenic glycosides contents of both samples were however the same. The correlation test carried out revealed that there was no significant difference between the anti-nutrient contents of both samples ($p>0.05$).

Table 2: The metallic and non-metallic minerals composition of the production source sample of cashew nuts and the market sample.

Metallic minerals	Production Source Sample Conc. (Mg/Kg) Mean ± SD	Market Sample Conc. (Mg/Kg) Mean ± SD
Na	9.54±0.02	15.46±0.22
Mg	52.96±0.012	42.16±0.003
Ca	5.04±0.063	3.93±0.003
Mn	0.40±0.24	0.053±0.12
Zn	0.47±0.0029	0.55±0.02
Cu	0.52±0.003	0.31±0.01
Cd	ND	ND
Pb	ND	ND
Phosphate	45.39 ± 0.01	45.38 ± 0.005
Nitrate	22.10 ± 0.02	44.14 ± 0.014
Nitrogen	0.28 ± 0.01	0.35 ± 0.012
Phosphorus	14.79 ± 0.01	14. 80 ± 0.01

($p>0.05$)

Table 3: The anti-nutrient contents of the production source sample and the market sample of cashew nuts.

Anti-nutrient composition	Producrion Source Sample Conc. (Mg/Kg) Mean ± SD	Source Sample Conc. (Mg/Kg) Mean ± SD
Alkaloid	9.78 ± 0.01	9.55 ± 0.02
Flavonoid	3.03 ± 0.015	3.45 ± 0.02
Saponin	9.04 ± 0.02	9.04 ± 0.01
Cynogenic glycoside	0.021 ± 0.0005	0.021 ± 0.001

($p>0.05$)

Discussion

The result of isolation of fungi associated with the nuts from source of production and the market showed that *Claviceps purpurea*, *Aspergillus fischeri*, *Aspergillus carbonarius*, *Aspergillus flavus*, *Aspergillus tamrii* and *Aspergillus fumigatus* were associated with seed samples from the market and source of production. *Aspergillus flavus*, *A. tamari*, *A. carbonarius*, *A. fischeri*, *A. fumigatus* and *Claviceps purpurea* [13]. The proximate composition (%) of the source of production and market samples of cashew nut studied are shown in Table 1. From the data, it was observed that the production source sample contained moisture content (1.68 ± 0.003), while market sample contained (4.91 ± 0.04). The moisture content of both samples was low when compared to moisture content of most legumes usually between 7.0 and 11.0% as reported [18]. However, these values are in close agreement with those reported [19] for fluted pumpkins seed of 5.0%. It should be known that seed with low moisture content should store for a longer time without spoilage. The ash content mean value of cashew nut in this present study was 2.92 ± 0.04 and 2.13 ± 0.015 for source and market samples respectively. Previous studies showed ash content of kola nut, jack bean and cowpea to be 3.1%, 3.6% and 3.2% respectively [20] and of cashew nut flour 4.4 ± 0.1% [21]. The ash content of 1.5-2.5% for nuts has been recommended for suitability as animal feeds [22], but with the value of ash reported in this study; market sample of cashew nut may be suitable for animal feed, while source sample of cashew nut may be unsuitable. This could have been caused by the action of fungi on the long exposed market sample. This is partially in agreement [21].

The protein content of source sample 24.85 ± 0.003 was high in comparison with protein rich foods such as soybeans, cowpeas, pigeon peas, melon, pumpkin and gourd seeds ranging between 23.1-33.0% [24]. The recommended daily allowance for protein for children ranges from 23.0-36.0g and for adult, 44-56g [23]. However, it could be evaluated that cashew nut could supply the recommended daily intake of protein for children. It should be noted that pre-disposed market retailer sample of cashew nut in this study have low protein content of 17.52 ± 0.003. It could be suggested that the decrease in the protein content of the market sample might be due to utilization of some of the digested nutrient contents of the market sample by the storage fungi. Since their storage periods have lasted longer than those of the source sample. The longer storage period might have created a favourable environment for the invasion and infection of the market sample leading, to the breakdown of the nutrient content which the fungi utilized. The fibre content (11.70 ± 14 and 21.15) was very high when compared with legumes, the mean values of which range between 5-6% [21,25]. Maintenance of internal distention for a normal peristaltic movement of the intestinal tract is the physiological role which crude fibre plays. Okon (1983) reported that a diet low in fibre is undesirable, as it could cause constipation; as such diets have been associated with diseases of colon like piles, appendicitis and cancer [28]. The value obtained for carbohydrate (2.64 ± 0.08 and 8.48 ± 0.09) for both samples respectively are moderately low compared to the expected range of mean values for legumes (20-26%) of dry weigh [18]. Agbor (2008) had reported that very low carbohydrate diets might lead to increased cortisol levels which might cause some cancers, among other diseases. The increases in moisture content of the market sample of cashew nut as observed in this study could be attributed to the digestive, dissolution or solubilization of the nut tissue by the fungi causing the watery rot of the nut and of course leaving behind mostly fibre which might be the reason for the increased fibre content of the nut. As shown in Table 2, the Na and Zn contents were relatively higher in the market sample. This could be due to the conversion of the organic matter content of the nuts into inorganic ions leading to an increase in the Na and Zn contents of the cashew nuts in the market sample during degradative activities of fungi on the nuts. This is in accordance with, who stated that fungi require minerals such as Nitrates magnesium, calcium etc for growth [26]. The utilization of some of these minerals by fungi for their growth suggested that minerals deficiency disease could occur, as humans eat these nuts. For instance, calcium deficiency could lead to rickets and osteoporosis [27]. A correlation test carried out showed that there were no significant difference ($P > 0.05$) in the mineral composition (metallic and non metallic) of both samples of cashew nuts investigated.

The anti-nutrient contents of the source and market samples of *A. occidentale* as shown in Table 3 revealed that there was an increase in the alkaloid and tannin contents of the production source when compared with the market source. This agrees with [26], that anti-nutrients occur in relatively high levels in healthy plants where they have been known as major determinants of plant resistance to plant pathogens. It could, therefore, be deduced that the relatively high level of alkaloids in the production source might have been be responsible for their apparently healthy nature. The flavonoid content of the infected nuts which were relatively higher in the present investigation suggests that the source sample must have produced flavonoids as a point to their active response or defensive mechanism against the fungal attack. A correlation test carried out to determine if there is a statistical significant difference in metallic minerals, non-minerals and the anti-nutrient contents between the source and market sample of *A. occidentale* used in the present study revealed that the results showed no difference ($P > 0.05$).

Conclusion

The present study indicated that cashew nut is rich in important food properties compared to some other oil seeds and nuts. The high quality protein of the cashew nut showed its reliability as a good source of amino acids for children and adults. On the effect of fungi, it's advisable that proper storage means be employed to discourage high humidity that favours the growth of fungi, especially, the opportunistic ones. This is because, fungi utilizes the available nutrient for their growth thereby reducing the nutritive value of the cashew nut. They also secrete toxins that are injurious to human health.

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