International Journal of Research in Agricultural Sciences
Volume 2, Issue 4, ISSN (Online): 2348 – 3997)

Changes in the Nutrient and Anti-Nutritional Composition in Two Varieties of Cocoyam (Var *esculenta* and Var. *antiquorum* (L) Schott) Caused by Isolated Fungi Species in Plateau State, Nigeria

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Abstract **– Biochemical analyses were carried out to determine the changes that occurred in two varieties of cocoyam (var. *esculenta* and var. *antiqourum* (L.) Schott (Hubbard & Render) corms and cormels inoculated with *Alternaria alternate, Fusarium oxysporum, Verticilium lateritium, Botrydiplodia theobramae, Colletotricum coccoides, Phythium myriotylum, Fusarium verticillioides, Rhizopus stolonifer* and *Geotricum candidum*. Significant increases (P<0.05) were observed in moisture, protein, crude fat, fiber and ash contents of the two varieties of the cocoyam corms and cormels inoculated with the fungi during the 14-day period of incubation, when compared with the un-inoculated corms and cormels. In the var. *esculenta, A. alternata, B. theobromae, P. myriotylum, F. stolonifer, F. verticillioides, F. oxysporium, G. candidum, C. coccoides* and *V. lateritium* increased the moisture content, protein content,crude fat content,fiber content and ash content in corms and cormels incubated for 14 days in complete darkness at 25° C ± 2 C, when compared to their controls. The nine fungi caused a significant (P<0.05) reduction in carbohydrate and in vitamin content when compared to their controls. Similarly, in the var. *antiquorum, A. alternata, F. oxysporium, F. stolonifer, P. myriotylum, B. theobromae, P. myriotylum, C. coccoides, V. lateritium, G. candidum* and *F. verticillioides* increased the moisture content, protein content, crude fat, crude fiber and ash content when compared to the controls. The nine fungi caused a significant (P<0.05) reduction in carbohydrate and in vitamin contents. Vitamin composition showed the highest rate of decrease in var. *antiquorum* 0.10%-0.02% than var. *esculenta* 0.11%-0.05% inoculated with the same fungus (*V. lateritium*) compared to the control and the ones observed in both healthy varieties. The result revealed the presence of anti-nutritional composition of phytates, oxalates and cyanite in mg/l00g in both varieties, even though they both differed in concentration and thus, there was no significant difference (P>0.05) between them. The composition of oxalate is a little higher in var. *esculenta* than var. *antiquorum* with (2.53% and 2.17%) respectively. Moisture, protein and crude fiber contents increased more in var. *esculenta* than var. *antiquourum*, while crude fat and ash contents increased more in var. *antiquorum* than var. *esculenta* due to the effects of the fungi. Similarly, the fungi caused a higher decrease in the carbohydrate content of var. *esculenta* than var. *antiquorum* . The results of the study showed that the presence of fungi in cocoyam causes significant changes in the nutritional and biochemical components of the corms and cormels.**

Keywords **– Fungi, Cocoyam Corms and Cormels, Nutritional Composition, Nigeria.**

I. INTRODUCTION

Cocoyam belongs to the monocotyledonous family *Araceae* (the aroids) which contains several plants which are cultivated and used for food in various parts of the tropics [29]. They are mainly herbaceous plants, often with an enlarged rootstock, which acts as a storage organ. Morphologically, the primary corms of the plants represent the main stem and the secondary corms or cornels, lateral branches. The relative sizes of the two types of organ vary greatly between species and variety - the cornels are usually the more satisfactory item of food. Cocoyam (*Colocasia esculenta* L. Schott) belongs to the genus; *Colocasia*, and generally comprised of a large spherical corm (swollen underground storage stem), from which a few large leaves emerge [29]. It refers to the two members of the Araceae family that are staple foods for many people in developing countries in Africa, Asia and the Pacific [3], namely *Colocasia esculenta* (L) schott and *C. antiquorum* (L) schott. It is an important tropical tuberous crop, and a traditional starch staple food for millions of people in the developing countries of the tropics, subtropics, Pacific Islands, West Indies and the Mediterranean [29]. Taro is considered a very polymorphic species with at least two botanical varieties [31].

Botanically, the genus *Colocasia* has two important varieties; those with relatively small corms surrounded by large cormels often referred to as 'eddoe' classified as *Colocasia esculenta* var.*antiquorum* (L.) Schott (Hubbard and Rehder) and those with large cormels sometimes called 'dasheen', classified as *Colocasia esculenta* var.*esculenta L.* Schott [11], [18]. These two varieties are mainly differentiated by the shape and size of the main corm and cormels. Var. *esculenta* genotypes have a larger main corm and smaller side cormels whereas var. *antiquorum* genotypes usually have a relatively smaller central corm and well-developed side cormels [29], [30], [31].

In Northern Nigeria, Plateau State to be precised, the 'eddoe' and 'dasheen' species of cocoyam are traditionally called 'Tsohon gwaza' and 'Sabon gwaza' respectively. The corm produces lateral buds, which gives rise to tubers or cormels and suckers or stolons.

Nutritionally, cocoyam is rich in carbohydrates (13-29%), vitamins and minerals. It also contains proteins (1.4

- 3.0%) the leaves are rich sources of vitamin B6, vitamin C, niacin, thiamin and riboflavin [21], [29]. They are also rich in minerals such as iron, phosphorus, zinc, potassium, copper and manganese. In addition to starch, the corms and cormels are good sources of starch, dietary fiber (0.60 -1.18) as well as oxalic acid, which cause serious irritation when raw corms come in contact with the skin [11]. The high digestibility and the very small size of the starch granules make cocoyam a very suitable base for baby's food [25]. Although cocoyam composed predominantly of starch, it is next only to certain varieties of yam in crude protein content among root crops. It has appreciable quantities of calcium and phosphorus. *Colocasia* starch grains are among the smallest found in the plant kingdom; hence it is very easily digested and can form a good base for formulating infant food.

The need to achieve food security in Nigeria has generated increased interest in research, production and consumption of cocoyam. According to [19], Nigeria is the largest producer of cocoyam in the world, accounting for about 37% of the world's output estimated to annual production of 5.49 million metric tons, followed by Ghana which producers 31%. Cocoyam production in Nigeria is labour intensive with most operations carried out manually at the traditional level. Some of the advantages of cocoyam cultivation are that it has no strong obstructing stems as in cassava *(Manihot spp*), it has no vines to stake as in yams *(Discorea spp)*, and no entangling vines like sweet potato *(Ipomea* spp), [24].

Moreover, it is widely perceived that cocoyam production and processing in the country does not keep pace with other major root and tuber crops [6], this is believed to be attributed to its declining yields, low storability and the socio-cultural perception of the crop as women's crop, as women do not have control over land, labour and capital in some parts especially South -eastern Nigeria [14], [18], [33]. This is worsened by the devastating diseases caused by the fungi which are the major threats to cocoyam production [10], [22], [26].

However, the nutritive value of the two varieties and their contribution to human nutrition has been threatened by the presence of fungi species during storage, thus affecting the nutritional value of the varieties and possible commercial uses of the food. A more detailed study of the proximate and biochemical compositions of the diseased samples as well as the anti-nutritional factors was considered necessary to determine the above mentioned factors.

II. Materials and Method

Sample Collection.

Fifty rotten and healthy corms and cormels of the two varieties of cocoyam (*Colocasia esculenta* var. *esculenta* and *Colocasia esculenta* var. *antiquorum* (L) schott) were purchased randomly from six markets in two local government areas (Mangu and Bokkos) that are major producers of cocoyam in Plateau state.

Isolation of Fungal Pathogens from Rotten Cocoyam Corms and Cormels.

The isolation of fungal pathogens was done using the methods of [12] and [1]. Twelve samples each of the two varieties collected were surface sterilized with one per cent (1%) Sodium hypo chloride (NaCIO) solution for one minute to remove surface contaminants after washing off soil and roots debris or extraneous materials from the corms and cormels. This was followed by three successive rinses in sterilized water. After seven days, rotten corms and cormels showing inherent diseased symptoms of soft or white, black, brown and hard rots were removed as diseased samples. They were later categorized using different codes; as soft rot (SR), brown rot (BR) and hard or dry rot (HR) respectively for easy identifications. The diseased samples were used for the isolation of fungal pathogen. Diseased portions or areas where the presence of rots was apparent were cut out using sterile kitchen knife and sliced into small pieces (l-2mm diameter) separately according to the diseased symptoms earlier observed physically on the corms and cormels. The cormel piece were placed on sterile paper towels in a Laminar Air flow Hood chamber for 10 minutes to dry and then placed on to sPDA plates using a sterile inoculating loop. The plates were incubated at 27°C for seven days and then examined daily for the development of fungi growth. Each experiment was replicated six times for each of the identified physical diseased symptoms.

Sub Culturing/Purification and Identification of Test Fungi Pathogens.

When growth has established, subcultures were prepared using inocula from the different organisms in the mixed cultures to obtain a pure culture, this was done by transferring hyphal tips from the colony edges of the mixed cultures to fresh plates of sPDA using flame-sterilized blades. After sub-culturing the plates were incubated at 27°C until pure cultures were obtained. Seven day old cultures of the fungi from the two varieties of the cocoyam were used according to their diseased symptoms. Wet mount of the pure cultures were prepared and mounted on a drop of lacto phenol in cotton blue stain and placed at the center of a clean slide. The resulting pure cultures were used for characterization and subsequent identification of the fungi isolates with the aid of a compound microscope and a bi-nuclear (in order to get a very clear sporangia and sporangiospores) at ×40 and ×100 objectives for every diseased symptom identified physically and structures which include micro and macro conidia, sporangia, septation etc. where observed and compared with standard published identification guides by [15], [16], [17] and [32].

Pathogenicity Test.

This test was conducted to determine the ability of each fungi isolate to cause disease on fresh apparently healthy cocoyam corms and cormels. Fresh healthy whole corms and cormels of the two cocoyam varieties were first washed with tap water and thereafter dipped completely in 1% sodium hypochlorite (NaOCl) solution. The corms and cormels were placed on sterile paper towels and allowed to dry for 12minutes in a Laminar Air flow hood. Sterile cork borer (3mm diameter) was used to bore holes in the cocoyam cormels. The parts of the cormels which were

bored out at each point were kept in sterile Petri dishes. An agar block measuring 4mm by 4mm from growing cultures of each test isolates (pure cultures) was inoculated into the hole made with the aid of another cork borer (4mm diameter), after the inoculation the parts of the corms and cormels bore out were carefully replaced and sealed with sterile Vaseline to prevent contamination and labeled accordingly. A control experiment which bore no isolate was also setup (inoculated with 1ml of sterile distilled water). After inoculating the entire test isolates into their respective healthy corms and cormels, all the cormels and corms were incubated for 14 days in a humidity chamber. The corms and cormels were examined daily for evidence of rot such as softening, discoloration and offensive odour. At the end of the 14 days incubation period, the corms and cormels were carefully cut open along the line of inoculation to expose the regions of the corms and cormels which were then examined for rot. Where positive, the length and girth of the rot area and those of the entire corms and cormels as shown were measured and recorded.

Biochemical Analysis

Determination of the proximate composition of the sample was based on [9] procedures, employing the micro-kjeldahl method for crude protein and lipid/fat or ether was determined using the principles of the Soxhlet method outlined by [13]. Moisture, fiber and ash contents were determined as described by [9]. The carbohydrate was determined by difference, vitamin content was determined using the spectrophometric method of vitamin C determination, which involves the use of di-nitrophenyl hydrazine (DNPH).

Data Analysis

Data obtained by measurement of radial diameter were analyzed using T-Test and analysis of variance (ANOVA), the rate of growth of inoculated corms and cormels of each variety and the means were separated with Duncan's Multiple Range Test (DMRT).

III. RESULTS

The result of the biochemical analysis for the fungus-inoculated cocoyam corms and cormels and uninoculated controls in the two varieties incubated for 7-14 day-intervals are presented in Tables 1 and 2, for the var. *esculenta* and var. *antiquorum*, respectively. The fungi caused appreciable changes in the food components of the two varieties. Moisture, protein, crude fat, fiber and ash contents showed significant increases ($P<0.05$) in the corms and cormels inoculated with *A. alternata, F. oxysporium, F. stolonifer, P. myriotylum, B. theobromae, P. myriotylum, C. coccoides, V. lateritium, G. candidum* and *F. verticillioides* when compared to the un-inoculated corms and cormels (Table 1 and 2). The carbohydrate and vitamin contents decreased significantly ($P<0.05$), when compared to the uninoculated or controls (Table 1 and 2). In the var. *esculenta* corms and cormels inoculated with *V. lateritium* recorded the highest increase in fiber and ash contents (4.39%, 2.98%), while those inoculated with *P. myriotylum* and *F. oxysporium* recorded the highest increase in moisture content (99.00% and 94.50% respectively). Similarly, corms and cormels inoculated with *F. verticillioides* and *V. lateritium* recorded the highest increase in protein of 6.94% and 6.59% respectively. The lowest decrease in carbohydrate and vitamin contents were recorded in corms and cormels inoculated with *F. verticillioides* and G. *candidum* (07.21%, 0.04%). In var. *antiquorum*, corms and cormels inoculated with *B. theobromae* recorded the highest increase in moisture and protein contents (83.70%, 3.99%). Corms and cormels inoculated with *V. lateritium* recorded the highest decrease in carbohydrate and vitamin contents (11.02%, 0.02%). Moisture and fiber contents of the infected var. *esculenta* corms and cormels increase more than the infected corms and cormels of var. *antiquorum*, while ash and crude fat contents of the infected corms and cormels of var. *antiquorum* increase more than the infected corms and cormels of var. *esculenta* (Table 1 and 2), In the same vein, the carbohydrate content of the infected corms and cormels of var. *esculenta* decrease more than the infected corms and cormels of the var. *antiquorum*, while the infected corms and cormels of the var. *antiquorum* showed more decrease in vitamin content, compared to the corms and cormels of var. *esculenta* (Table 1 and 2).

Table 1: Changes in the Level of Nutrients of var. esculenta cocoyam Inoculated with eight (9) Selected Fungal species and Incubated at 25° C±2° C for 7-14 days.

Nutritional composition (% w/w)	Control		*A.alternata*		*B.theobromae*		*P.myrioiylum*		*R. stolonifer*		*F.verticillioides*		*F.oxysporium*		*G.candidum*		*C, coccoides*		*V.lateritmm*	
	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14
Moisture	73.88	73.86	73.89	89.91	73.90	87.98	78.90	99.02	84.32	98.46	74.36	88.52	84.4	94.5	73.83	94.26	74.8	82.8	74.7	84.9
Carbohydrate	17.16	17.14	16.94	14.18	13.1	10.98	12.12	09.52	11.28	08.94	11.32	07.21	14.53	12.92	11.39	07.69	09.38	04.94	10.94	09.82
Protein	4.66	4.70	4.76	4.99	4.78	5.73	4.76	5.89	4.71	5.02	4.81	6.94	4.89	5.87	4.94	6.51	4.83	5.93	4.71	6.58
Crude fat	0.20	0.26	0.32	0.48	032	0.57	0.29	0.87	0.50	0.97	0.42	0.89	0.36	0.79	0.56	0.98	0.39	0.77	0.32	0.61
Fiber content	2.90	2.98	2.96	3.34	2.96	3.11	2.94	3.22	2.91	3.98	2.99	3.83	2.96	3.07	2.91	4.13	2.99	4.06	3.02	4.39
Ash Content	1.20	1.24	1.25	1.30	1.26	2.04	1.25	1.35	1.25	1.48	1.25	2.40	1.42	1.93	1.59	2.77	1.48	2.91	1.65	2.98
Vitamin	0.14	0.14	0.13	0.07	0.13	0.09	0.11	0.07	0.10	0.05	0.06	0.04	0.10	0.04	0.09	0.04	0.12	0.08	0.11	0.05

The values are mean ±SD of six determinations. Values that are not showing a common superscript letter in a column are significantly different at $P<0.05$ as assessed by Duncan's multiple range tests.

Table 2: Changes in the Level of Nutrients of var. antiquorum cocoyam inoculated with eight (9) selected fungal Species and Incubated at 250 C 20 C for 7-14 days.

Nutritional composition (% w/w)	Control		*A.alternata*		*F.oxysporum*		*R.Stolonifer*		*P.myriotylum*		*B.theobromae*		*C.coccoides*		*V.lateritium*		*G.candidum*		*F.vertcillioides*		
	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	14
Moisture	69.02	69.02	69.1	69.53	70.12	79.88	64.21	80.45	71.19	71.86	73.21	83.70	73.3	82.58	74.37	75.4	71.620	81.6	70.56	80.16	72.08
Carbohydrate	23.82	21.82	20.74	19.60	19.56	21.11	19.20	11.12	21.74	18.60	22.28	22.09	20.32	16.40	22.85	11.02	19.401	12.5	17.34	11.45	23.08
Protein	2.72	2.72	2.76	2.82	2.98	3.58	2.72	3.28	2.38	2.46	3.13	3.99	2.53	3.75	2.75	3.59	3.20	3.82	3.43	3.79	3.79
Crude fat	0.14	0.14	0.21	0.97	0.28	0.99	0.47	0.83	0.50	1.32	0.61	1.03	0.61	0.98	0.99	1.23	0.98	1.01	0.78	1.03	1.10
Fiber Content	2.90	2.90	2.96	2.99	3.90	3.06	2.98	3.69	2.96	3.60	2.99	2.97	3.13	3.83	2.94	3.24	2.97	3.40	2.98	3.09	4.78
Ash Content	1.40	1.42	1.43	1.94	1.58	2.75	1.78	2.02	1.78	2.06	2.03	2.63	2.04	2.49	1.61	3.08	1.60	2.54	1.89	3.01	2.96
Vitamin	0.20	0.20	0.20	0.19	0.17	0.10	0.17	0.08	0.17	0.12	0.19	0.16	0.19	0.09	0.10	0.02	0.19	0.12	0.19	0.13	0.2

The values are mean ±SD of six determinations. Values that are not showing a common superscript letter in a column are significantly different at P<0.05 as assessed by Duncan's multiple range tests.

T-test Comparison Between Healthy and Diseased Nutritional components of cocoyam varieties.

Comparison between healthy and diseased nutritional components of the cocoyam varieties indicated that moisture, protein, crude fat, ash and fiber contents increased as a result of the growth of the fungi from 69.02 ± 0.006 to 69.48±0.004, 2.72±0.006 to 3.66±0.009, 0.14±0.006 to 0.59±0.010, 1.40±0.006 to 1.75±0.005 and 2.90±0.008 to 3.90±0.006 in var. *antiquorum* respectively, while var. *esculenta* recorded a similar increase of 71.96±0.006 to 73,88±0.005, 4.66±0.006 to 6.53±0.008, 0.20±0.007 to 0.42±0.008, 1.20±0.004 to 1.25±0.007 and 2.90±0.004 to 4.90±0.007 respectively. This was significantly (P<0.05) different from the mean comparison of these nutrients observed in var. *esculenta* (Tables, 3, 4, 5, 6 and 8). On the other hand, carbohydrate and vitamin contents of the two cocoyam varieties decreased drastically after the action of the isolates on the corms and cormels of the healthy cocoyam varieties from 23.82±0.006 to 22.62±0.005 and 0.23±0.006 to 0.21±0.007 in var. *antiquorum*, which showed a significant (P<0.05) difference from 17.16±0.006 to 14.94±0.005 and 0.41±0.009 to 0.38±0.008 recorded in var. *esculenta* (Table 7 and 9) respectively.

Table 3: T-test comparison between moisture of healthy and diseased var. *antiquorum* and var. *esculenta*

Cocoyam Varieties	**Group**			
	Healthy	**Diseased**		**P –value**
Var. *antiquorum*	69.02 ± 0.006	69.48 ± 0.004	-63.3	<0.001
Var. *esculenta*	71.96 ± 0.006	73.88 ± 0.005	-252.2	<0.001

Table 4: T-test comparison between protein of healthy and diseased and var. *esculenta*

Cocoyam Varieties	**Group**			
	Healthy	**Diseased**	**T –statistic**	**P –value**
Var. *antiquorum*	2.72 ± 0.006	3.66 ± 0.009	-88.3	<0.001
Var. *esculenta*	4.66 ± 0.006	6.88 ± 0.008	-187	<0.001

Table 5: T-test comparison between crude fiber of healthy and diseased var. *antiquorum* and var. *esculenta*

Cocoyam Varieties	**Group**			
	Healthy	**Diseased**	**T –statistic**	**P –value**
Var. *antiquorum*	2.90 ± 0.008	3.90 ± 0.006	-98.8	<0.001
Var. *esculenta*	2.90 ± 0.004	4.90 ± 0.007	-258.2	<0.001

Table 6: T-test comparison between crude fat of healthy and diseased var. *antiquorum* and var. *esculenta*

Cocoyam Varieties	**Group**			
	Healthy	**Diseased**	**T –statistic**	**P –value**
Var. *antiquorum*	0.14 ± 0.006	0.59 ± 0.010	-39.6	<0.001
Var. *esculenta*	0.20 ± 0.007	0.42 ± 0.008	-20.45	<0.001

Table 7: T-test comparison between N.F.E (carbohydrate) of healthy and diseased var.*antiquorum* and var. *esculenta*

Cocoyam Varieties	**Group**			
	Healthy	**Diseased**	**T –statistic**	**P –value**
Var. *antiquorum*	23.82 ± 0.006	22.62 ± 0.005	150.40	<0.001
Var. *esculenta*	71.16 ± 0.006	14.94 ± 0.005	28	<0.001

Table 8: T-test comparison between Ash of healthy and diseased var .*antiquorum* and var *esculenta*

Cocoyam Varieties	Group			
	Healthy	Diseased	T –statistic	P –value
Var.*antiquorum*	1.40 ± 0.006	1.75 ± 0.005	-45.19	<0.001
Var. *esculenta*	1.20 ± 0.004	1.25 ± 0.007	-5.89	<0.001

Table 9: T-test comparison between vitamin of healthy and diseased var. *antiquorum* and var. *esculenta*

Cocoyam Varieties	Group			
	Healthy	Diseased	T –statistic	P –value
Var.*antiquorum*	0.23 ± 0.006	0.21 ± 0.007	-18.7	<0.001
Var. *esculenta*	0.41 ± 0.009	0.38 ± 0.008	-38.4	<0.001

IV. DISCUSSION

Inoculation of the cocoyam corms and cormels with pure cultures of *A. alternata, F. oxysporium, R. stolonifer, P. myriotylum, B. theobromae, P. myriotylum, C. coccoides, V. lateritium, G. candidum* and *F. verticillioides* incubated for 7-14 days at 25± 2 C resulted in various changes in the nutrients of the cocoyam varieties. These fungi were identified by [7] on some cocoyam varieties as the major fungi affecting cocoyam corms. The increase in moisture could probably be due to the fungal growth characteristics in using some of the cocoyam tissues as nutrients, and as a result, produces moisture in the process. This conformed to similar results obtained by [8] on sunflower seeds, [23] on cocoyam and [36] on groundnut seeds.

Vitamin content showed the highest rate of decrease in the two varieties used with 0.20%-0.02% and 0.14%-0.04% in var. *antiquorum* and var. *esculenta* respectively which was significant (P<0.05) different when compared to the control. A decrease in vitamin content could be due to the increase in moisture as a result of the growth of the fungi causing the vitamin to dissolve in it since it is a water soluble vitamin. Most species of the fungi isolated, especially the fast growers are most often deficient in thiamine and biotin, and thus they must obtain these vitamins from the environment and appeared able to synthesize all other vitamins necessary for their growth and reproduction [20]. This result agrees with similar researches by [5] on garden egg, [6] on cocoyam. Reference [2] also reported that the protein content of raw taro decreases after drying and processing, from 4.5% to 2.3%, [4] on Oca (*Oxalis tuberosa*), [35] on taro (*Colocasia esculenta*) and [34] on cocoyam (*Xanthosoma sagittifolium*) accessions. The increase in crude fat (Ether extract) could be due to decrease in total oil as a result of hydrolysis as proved by [27] and [28] on separate work on stored grains and palm produce respectively. The increase in the fiber content could be due to the presence of certain minerals elements like phosphorous and potassium in mycelia of the test fungi. The increase in the fiber content observed might have resulted from the removal of moisture during drying which increase the concentration of fiber. The result of this present study showed that the fungi isolated caused deterioration of the cocoyam varieties and alters the nutritional and biochemical value of the corms and cormels.

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