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Effect of Storage Moulds on the Nutritional Quality of Kolanuts in Nigeria

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Abstract: Proximate analysis of healthy and infested kolanuts (*Cola nitida* and *C. acuminata*) was carried out at the Cocoa Research Institute of Nigeria, Ibadan, Nigeria, to determine the effect of mould attack on the nutritive value of stored kolanuts. The storage moulds encountered on both species of kolanuts in Nigeria were *Lasiodiplodia theobromae, Fusarium pallidoroseum, F. moniliforme, F. cavispermum, Aspergillus niger, A. fumigatus, A. flavus,* and *Paecilomyces variotii*. Proximate analysis of healthy and infested *C. nitida* revealed 0.79% and 1.29% soluble sugar in healthy and infected kolanuts respectively. The healthy *Cola acuminata* has 54% moisture content compared to 23.24% in the infested nuts. The percentage of crude protein in healthy nuts was 4.55% but it was 2.1% in the infested nuts. Macro elements of the kolanuts indicated significant differences in calcium, potassium, magnesium and phosphorus between healthy and infested nuts. There was an increase in caffeine content of diseased *C. nitida* and a significant decrease ($p=0.05$) in percentage protein and moisture content in such infested kolanuts. The activities of the isolated mould are implicated in the deterioration of these biochemical substances in kolanuts.

Key words: Storage mould, kolanuts, deterioration, nutritive value

INTRODUCTION

Cola popularly called kolanut is indigenous to the tropical rain forest of West Africa, West Indies, Brazil and Java (Rusell, 1955). *Cola nitida* and *C. acuminata* are the most common *Cola* species and *Cola* of commercial value in Nigeria. The effects of chewing the odourless nut with an astringent taste have enhanced its continued use for about 1000 years now in Nigeria (Agatha *et. al.*, 1978). The habit if not comparable to the tobacco smoking of Western civilization or opium usage of far Eastern societies certainly shows similarity to the Betel nut chewing of Asiatic communities (Agatha *et al.*, 1978). Outside West Africa, cultivation and trade in kolanut have been established in countries like the Caribbean Islands, Mauritius, Sri-Lanka and Malaysia (Oladokun, 1982).

A few hundreds of tonnes of dried kolanuts are exported annually from West Africa to North America and Europe. These are used for the preparation of beverages (e.g. kola chocolate) and pharmaceutical products (e.g. laxatives). The presence of alkaloids and other chemicals in kolanuts such as caffeine, kolanin and theobromine also make them suitable for use in drug preparations (Anon, 1985; Olunloyo, 1979). The geographical and chronological spread in trade and use of kola has inevitably created a high demand for kolanut far in excess of its production.

Storage of kolanuts has always been a laborious and delicate task. For now, kola traders are still using traditional methods of storage. Much of the produce is chewed in its fresh state even though the bulk of the nut is harvested once in a year. Preserving the freshness of the nuts for many months without storage moulds is therefore a problem, which farmers and traders seek to solve.

A loss of up to 50% per tonne due to storage moulds may be recorded in poorly stored nuts (Olunloyo, personal communication). Kolanut traders often control spoilage by removing infested nuts at intervals during the storage period and throw them away. Thus, the specific objective of this present study was to evaluate the nutritional quality of stored kolanuts through proximate analysis with view of finding alternative uses for infested kolanuts.

MATERIALS AND METHODS

Healthy and infested kolanuts (*Cola nitida* and *C. acuminata*) collected randomly during processing and storage from various locations in Nigeria were used in this study. The culture media used for the fungal isolations and counts were potato dextrose agar (PDA) (Difco, Detroit, USA). The media were sterilized by autoclaving for 15 minutes at $1.05 kg/cm^2$ ($121^{o}C$).

Analysis: Healthy and infested kolanut samples were assayed for the percentage moisture content using the oven-dried weight method. Fresh samples of kolanut were dried to constant weight in an air oven at $105^{o}C$ for 15 hours and the percentage moisture content was calculated as described by Agrawal (1980); AOAC (1990).

The ash content of healthy and infested kolanuts was determined using the method described by Osborne and Voogt, (1978). Fresh samples (10g) of kolanut

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Table 1: Fungal species isolated from *C. nitida* and *C. acuminata*

Genus	*C. nitida*	*C .acuminata*
L. theobromae	+	-
F. pallidoroseum	+	+
F. moniliforme	+	+
F. cavispermum	+	-
A. flavus	+	+
A. niger	+	+
A. fumigatus	-	+
P. chrysogenun	+	+
P. variotii	+	+
Chlamydomyces sp	+	-
Curvularia sp	+	-
M. spinosus	+	+

Table 2: Proximate analysis of healthy and infested *C. nitida* samples

	Biochemical	Composition (%)
	Healthy nut	Infested nut
Moisture content	62.3±1.973	35.6±2.254
Crude Protein	1.36±0.03	0.37±0.02
Fat	0.77±0.10	0.74±0.05
Caffeine	2.1±0.161	2.52±0.002
Sugar	0.79±0.015	1.29±0.075
Ash	1.57±0.153	4.96±0.404

Values were expressed on a dry weight basis as the means ± s.d of 3 determinations.

Table 3: Proximate analysis of healthy and infested *C. acuminata* samples

	Biochemical	Composition (%)
	Healthy nut	Infested nut
Moisture content	54±2.30	23.24±1.23
Crude Protein	4.55±0.05	2.10±1.02
Fat	2.39±0.10	1.17±0.05
Caffeine	0.023±0.001	0.009±0.002
Fibre	11.47±2.015	6.13±1.75
Ash	3.43±0.4.53	1.92±0.340

Values were expressed on a dry weight basis as the means ± s.d of 3 determinations.

were put in pre-weighed crucibles and later in a furnace. The organic matter was burnt off and the inorganic material remaining was cooled and weighed. Heating was carried out in stages, first to drive off the water, then to char the nut samples thoroughly and finally to ash at 600°C in a furnace. The percentage ash content was calculated as described by A.O.A.C. (1990).

The Kjeldahl procedure was used for the determination of the total nitrogen in both healthy and infested nut samples. The amount of crude protein contained in the samples was obtained described by Osborne and Voogt, (1978); Tel and Hargathy, (1984).

The method of extraction of fat of *C. nitida* and *C. acuminata* was adopted from fat extraction methods of Egan *et al.* (1981). The kolanuts were ground using a ceramic pestle and mortar before blending in an electric Philips kitchen blender. An amount of 20g of the ground kolanut was packed into the extraction thimble before covering with a small ball of cotton wool. The thimble was inserted in a quick fit plain body Soxhlet extractor. Petroleum ether in the quantity of 200ml (60-80°C) was poured in a 250ml round-bottom flask of known weight, which was connected to the extractor, and refluxed on an electric thermal heater for 5 hours. The quantity of the fat extracted from the nuts was determined as described by Adekunle and Badejo (2002).

The extractable caffeine in both healthy and infected kolanuts were determined according to A0AC (1990). Samples comprising both healthy and infested kolanuts were analyzed for the calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), and phosphorus (P). The total amount of these minerals contained in a sample was determined by burning away the organic matter. Burning was carried out at a low temperature in order to avoid any loss of volatile mineral content. The individual mineral content of the kolanut sample was then determined as described by Tel and Hagarty (1984) using atomic absorption spectrometer.

RESULTS

Twelve storage rot fungi were isolated from each of infested *Cola nitida* and *Cola acuminata*. The fungi isolated are *L. theobromae, F. pallidoroseum, F. moniliforme, F. cavespermum, Curvularia sp., A. fumigatus, P. chrysogenum, F. cavispermum, M. spinosus, A. flavus, P. variotii, Chlamydomyces sp.* (Table 1).

The results of proximate analysis for both healthy and infested *C. nitida* and *C. acuminata* are shown in Table 2 and Table 3 respectively. The healthy kolanuts has 62.3% moisture content compared to 35.5% in the infested nuts (Table 2). The percentage caffeine was 2.1% in healthy nuts but it was 2.52% in infested nuts. Similarly, the level of sugar in the infected nuts was 1.29% whereas it was 0.79% in healthy nuts.

The percentage of crude protein in healthy nuts was 1.36% but it was 0.37% in the infested nuts. There was a significant difference (P = 0.05) in the values obtained for moisture content, crude protein and ash in both healthy and infected kolanuts.

No significant difference was recorded for values obtained for caffeine and fat contents. Perhaps the storage moulds could not easily degrade the caffeine and fat content of the infested nuts. From the results shown in Table 2, invasion of healthy kolanut by storage moulds generally affect the nutritive quality of stored kolanuts.

The mineral analysis of the healthy and infested *C. nitida* and *C. acuminata* nuts is shown in Table 4. There were variations in the mineral contents of the two species of *Cola*. The calcium content in *C. nitida* was estimated to be 0.69% and 0.417% for the healthy and infested nuts respectively. A similar trend of 0.731% and 0.562% respectively was recorded in *C. acuminata* for both healthy and infected nuts.

Table 4: Mineral Composition of both healthy and infected kolanuts

	C. nitida		C. acuminata	
Minerals	Healthy	Infested	Healthy	Infested
Copper (Cu)	0.04±0.01	0.02±0.004	0.13±0.18	0.03±0.01
Iron (Fe)	0.06±0.05	0.08±0.004	0.13±0.09	0.06±0.01
Potassium (K)	2.25±1.23	0.49±0.03	1.85±0.43	0.91±0.01
Magnesium (Mg)	0.29±0.01	0.24±0.026	0.41±0.21	0.44±0.01
Manganese (Mn)	0.02±0.01	0.05±0.004	0.05±0.02	0.05±0.01
Sodium (Na)	0.36+0.098	0.14±0.03	0.33±0.12	0.26±0.001
Phosphorus (P)	0.85±0.16	0.57±0.03	1.22±0.28	0.81±0.01
Zinc (Zn)	0.04±0.06	0.07±0.004	0.01±0.001	0.01±0.06

Values were the means ± S.D of the 3 determinations.

The mineral content in the two species of *Cola* is generally high and well distributed. However the mineral composition varied according to species (Table 4). The values obtained for calcium, potassium, phosphorus, magnesium and sodium in healthy *C. nitida* were higher than those obtained in infested nuts. However, potassium is highly concentrated in the healthy nuts of both *C. nitida and C. acuminata*.

The iron content was found to be lower in healthy kolanuts of *C. nitida* when compared with the content in the infested kolanuts. Similar trend was also observed for manganese and zinc (Table 4). However, the value obtained for copper was higher in healthy both *C. nitida* and *C. acuminata* than the value obtained in infected nuts.

A general view of the results revealed that the values obtained for copper, manganese and zinc for both *C. nitida and C. acuminata* were generally low. It is evident from the results presented in Table 4 that healthy kolanuts is rich in major elements such as calcium, potassium, phosphorus, sodium and magnesium. The activities of the storage moulds were implicated in the depletion of these major elements in the stored kolanuts.

DISCUSSION

Neegaard (1977) reported that seed-borne fungi are responsible for deterioration of food reserves in the seeds. As discovered in this study, the results of the proximate analysis of both healthy and infected kolanuts established the deterioration of food reserves in the infected kolanuts when compared to healthy nuts.

Similarly, effects of the storage moulds were also established in the mineral composition of healthy and infected *Cola nitida* and *Cola acuminata*. Reduction in carbohydrate level has been reported in several diseases of other crops (Sempico, 1959). Olofinboba (1968) found that *Botryodiplodia theobromae* cause great depletion in carbohydrate present in *Antiaris africana*; Ogundana *et al.* (1975) found that carbohydrate, protein and lipids were reduced in sweet potatoes infected by *Lasiodiplodia theobromae*. Stavely and Chaplin (1972) observed that tobacco leaves heavily infected by *Cercospora nicotianea* averaged 46% less reducing sugars and 6.5% more total nitrogen than non-infected leaves.

The present study demonstrated the changes due to infection by *L. theobromae and F. pallidoroseum* on the food content of kolanut in Nigeria. There was a decrease of 42.9% in moisture content on the infected nuts compared with healthy nuts. Similarly, there was a decrease of 54.4% in crude protein and fat contents in the infected nut when compared with the healthy nuts.

The results of the present studies corroborate the reports of other workers. Sharma and Bhowmik (1987) reported that fungal infection caused both quantitative and qualitative damages to groundnut (*Arachis hypogea*). Furthermore, the diseased kernels had lower oil percentage but higher ash content. Singh *et al.*, (1972) reported reduction in oil content of sesame (*Sesamum indicum*) seeds infected with *Macrophomina phaseolina*. Also, Lalithakumari *et al.* (1971) reported that groundnut seed-infecting fungi like *Aspergillus flavus*, *Cladosporium herbarum* and *Lasidiplodia* sp. caused alteration in the oil colour.

The present studies clearly showed increase in the content of reducing sugar in the diseased kolanut when compared to healthy ones. This may be due to the susceptibility of cellulose to enzymatic degradation of invading fungi. The large cellulose molecules are hydrolyzed by microbial enzymes into glucose and utilized by the degrading microorganisms (Siu and Reese, 1953; Whitaker, 1957).

Similarly caffeine and ash contents were found to be higher in infected nuts when compared to the healthy nuts. One explanation for this increase in caffeine content could be attributed to the net loss of the other components besides the moisture content. That the protein content of the healthy nut was higher than that of the diseased nuts, suggest the utilization of the nitrogen in the protein component by the pathogenic fungi. Conversely the total sugar in the infected nuts was higher that the quantity recorded in the healthy nuts. One explanation is that the plant, do store their carbohydrate in form of starch. Again, the fungi are well known to have

the ability to hydrolyze starch into reducing sugar. Consequently, higher value was recorded for sugar in the diseased nut. Previous works in support of this observation include those of Sharma and Bhowmik (1987). The values of protein contained in plant tissue may vary in terms of nitrogen content – from 13 percent to 18 percent Tel and Hagarthy (1984). Thus the 1.36% of protein content found in kolanut is very low when compared with 5.4% for oil seed protein, 5.9% for cereal protein and 6.6% for plant leaf protein as reported by Tel and Hagarthy (1984).
Studies have shown that the major elements such as calcium, potassium, phosphorus, sodium, magnesium and iron were well distributed in both *C. nitida* and *C. acuminata* nuts. However, the values obtained for calcium, potassium, phosphorus, magnesium and sodium in healthy C. *nitida* was higher than the values obtained in infested nuts. Conversely, the amount of copper, iron, manganese, and zinc were lower in healthy nuts than in the infested nuts. Thus, this study concluded on the note that storage moulds depreciate the quality of *C. nitida* and *C. acuminata*.

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