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Full Length Research Paper

Mycology and spoilage of retail cashew nuts

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Thirty-two samples of retail cashew nuts from Lagos, Nigeria were examined on two media. The pH values (5.1-6.3) of all the samples were conducive for fungal growth and mycotoxin production. Moisture content levels ranged between 4.1 and 6.8%. Fifteen samples had moisture contents up to or above 5.8%, the highest level estimated to be 'safe' for the storage of the nuts. Fourteen fungal species, mostly toxigenic and belonging to 5 genera were isolated. Seven species were from genus *Aspergillus,* 3 from *Penicillium,* 2 from *Rhizopus* and one each from *Mucor* and *Syncephalastrum.* The most predominant isolates were: *A. niger, A. restrictus, A. flavus*, *A. fumigatus* and *Aspergillus* sp. The mean and range of total fungal counts (CFU/g) in samples were: 3,368 (180 to 16, 300). At acceptable fungal levels of 10^3 and 10^4/g, only 14 and 28 samples, respectively, were deemed fit for human consumption. All the species recovered induced detectable loss in weights of the milled nuts, though to varying extents and would be expected to cause considerable spoilage of the nuts.

Key words: Cashew nut, *Anacardium occidentale*, fungal count, mycology, *Aspergillus* sp., *Penicillium* sp., spoilage.

INTRODUCTION

The cashew plant, *Anacardium occidentale* L, is a small to medium sized tree belonging to the family Anacardiaceae (Cobley and Steele, 1976). The fruit is a kidney-shaped achene about 3c m long with a hard grey-green pericarp. The seeds are the source of cashew nuts and they are normally removed from the pericarp after the fruits are roasted, a process which burns off shell oil and cooks the seeds.

Worldwide, cashew nuts are an esteemed and highly priced food delicacy because of their pleasant taste and flavour. The post-harvest processing, packaging and marketing have been commercialized and modern technology and regulations adopted in major producing countries like India and Tanzania. In Nigeria however, despite the cultivation of cashew in plantations and the establishment of cashew-processing factories (Esuruoso, 1974), peasant processing and packaging methods are still commonly adopted. The latter predisposes the nuts to mould contamination especially during hawking of the product, which usually are packaged in hand-knotted thin polyethylene bags. There are no labels to indicate vital information such as the name and address of producer, nutritional contents, recommendations for storage and the best-before- date for human consumption. However, mycotoxicoses are becoming increasingly implicated in human and animal pathology (Bacha et al., 1988). The situation may be worsened by consumers' reluctance to discard fairly mouldy food samples such as cashew nuts due to the irresistible taste and flavour.

This paper is a report on the mycology of retail processed cashew nut samples from the Lagos

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metropolis, Lagos State, Nigeria. The roles of isolates in the degradation, and thus spoilage of the nuts, were also determined.

MATERIALS AND METHODS

Samples

A total of 32 samples (each comprising 10 randomly picked consumer packs per retailer) were obtained from different locations in the Lagos metropolis. The physical conditions of the packaging material and of the nuts were carefully noted. On the day each sample was collected, the 10 composite packs were opened and the nuts were aseptically transferred into a big thick sterile polyethylene bag after which the content was thoroughly mixed together. The moisture content, pH analysis and mycological studies were also undertaken on the day of collection. Twelve youths with ages between 15 and 26 years were asked to chew without swallowing the nuts from each sample and report on the taste and crispness of the food.

Moisture content and pH analyses

Approximately 75 g portion of each sample was crushed in a mortar and the moisture contents of 5 g bits were determined after drying at 103^0C for 4.5 h. The pH values of 1:2 (w/v) suspensions of the pulverised material in distilled water were obtained.

Evaluation of visible mould incidence

Two hundred nuts were randomly obtained from a sample and carefully observed with the naked eye for any evidence of mould growth. The cotyledons (2 per nut) were then separated by hand and the state of the inner surfaces was also noted. The incidence of visible mould contamination was expressed as a percentage of the 200 nuts examined.

Mould incidence after surface disinfections

Forty cotyledons from the 200 nuts examined above were randomly picked and surface sterilised with a 2% aqueous solution of sodium hypochlorite for 2 min. This was followed by rinsing with three washes of sterile distilled water before four cotyledons were plated together equispaced from each on the two media used. Two of the cotyledons had their inner surfaces turned up and the remaining two had their outer surfaces turned up. Two media (Pitt and Hocking, 1985), of low water activity (a_w) were used. Malt extract agar with 40% (w/v) sucrose (MA40) and malt extract yeast extract 50% glucose agar (MY50G). Before pouring plates, chlortetracycline and chloramphenicol were added each at a concentration of 50 mg/L of medium. Plates were incubated upright at 28^0C for 5 to 8 days during which the number of cotyledons that yielded colonies was recorded. Also, the colonies were noted, enumerated and subcultured for identification. Fungal incidence was expressed as a percentage of the 40 cotyledons plated.

Mould incidence in non- disinfected samples

Another set of 200 nuts was randomly obtained from the sample with the aid of sterile forceps which were also used to split each of the nuts into two cotyledons. Without any surface sterilisation, 40 randomly picked cotyledons were plated and determination made as described above.

Estimation of total fungal counts

Randomly selected non-disinfected nuts (100) were pulverised and used for the study. The initial dilution was prepared by adding 10 g of sample to 90 ml diluent (40% (w/v) sucrose) and homogenised by stomaching for 2 min. Decimal dilutions (1 + 9 ml) were prepared down to 10^{-4}, and spread plated in triplicate, 0.1 ml per plate. MA40 that gave higher counts in a pilot study was the only medium used. Plates were incubated upright at 28^0C for 5 to 8 days.

Utilsation of cashew nuts by the isolated fungi

This study, following Adebajo (2000), was conducted to determine the participatory roles of isolates in the spoilage of the nuts. Fresh and healthy cotyledons were dry-milled, oven-dried at 90^0C to constant weight, mixed thoroughly and 25g portions dispensed into 250 ml conical flasks. The flasks were cotton-stoppered and wrapped in aluminum foil before autoclaving for 15 min at 121 ^{0}C twice in 24 h. Six ml of aqueous conidial suspension (10^6/ml) of 6-day old culture of a test fungus maintained on PDA slants was aseptically inoculated (using an atomizer) into the substrate in each flask. The latter was rotated during the process to ensure even distribution of the conidia-bearing mist. Each experiment was replicated five times. The approximate moisture content of the substrates after inoculation was 21.2%. Incubation was at 28^0C for 30 days after which the content of each flask was oven-dried at 90^0C for 48 h, cooled in a desiccator over anhydrous $CaCl_2$ and finally weighed. The difference in weight at the beginning and end of the experiment was due to fungal degradation.

RESULTS AND DISCUSSION

The moisture contents (%) recorded for the 32 samples ranged between 4.1 and 6.8 with a mean value of 5.4 (Table 1). More than half of the samples, that is 17 or 53% had moisture contents below 5.8%, which in a preliminary study conducted by the authors, was found to be approximately equivalent to 70% relative humidity (at the prevailing ambient temperature), which is generally considered the maximum level for safe storage (Pixton, 1982; Henderson, 1985). However, 15 or 47% of the samples had moisture contents up to or above the limit (5.8%) safe for storage and consequently, were predisposed to fungal growth and mycotoxin contamination during storage. Most stored agricultural products, including cashew nuts, being colloids, are hygroscopic i.e. they will absorb moisture from or give it up to the surrounding atmosphere until they are in equilibrium with it (Pixton, 1967). In the present study, the ease with which the thin container was perforated by the edges of the nuts suggests continuity in the environment inside and outside many of the retail packs. This leakage is expected to affect the overall quality and spoilage of the product. Indeed, research reports (Adebajo, 1992; Bankole et al., 1999; Desrosier and

Table 1. Moisture content (MC), pH, visible mould incidence (VMI) and total fungal count (CFU/g) in retail processed cashew nuts.

Sample No.	MC (%)	pH	VMI*	CFU/g
1	5.5	5.8	0	1,200
2	5.8	5.3	1.5	5,150
3	6.7	5.1	1.5	13,000
4	4.1	6.0	0	1,000
5	5.1	5.7	0	280
6	4.9	5.7	0	740
7	4.2	6.1	0	310
8	6.5	5.6	1	5,240
9	5.0	5.7	0	820
10	4.3	6.0	0	830
11	4.1	5.8	0	210
12	5.6	6.3	0	1,800
13	4.7	5.4	0	520
14	6.2	5.7	0	1,900
15	6.7	5.1	3	14,800
16	6.7	5.4	1.5	16,300
17	6.4	5.7	0	2,100
18	5.1	6.0	0	580
19	4.4	5.8	0	180
20	6.3	5.7	0	1,100
21	6.0	5.8	0	530
22	5.1	6.2	0	610
23	5.7	5.7	0	2,700
24	5.9	5.5	0	3,500
25	4.9	5.3	0	1,100
26	4.4	6.0	0	210
27	4.2	6.3	0	900
28	6.8	5.1	1	10,700
29	5.1	5.7	0	2,600
30	5.7	5.7	1	7,600
31	6.8	5.2	2	8,900
32	4.7	5.9	0	370
Mean	5.4	5.7	0.4	3,368

*In % of 200 nuts examined.

Desrosier, 1977; Frazier and Westhoff, 1978) on the influence of environmental conditions especially relative humidity and temperature on nuts and allied food products are well documented.

The presentations in Table 1 show that the mean pH and the range (in parenthesis) recorded for the samples were: 5.7 (5.1 to 6.3). Thus, the nut is a low acid food product and without exception, all the samples had pH values conducive to microbial growth and activities including the elaboration of toxic secondary metabolites (Frazier and Westhoff, 1978; Smith and Moss, 1985).

The visible mould incidence (%) estimates ranged between 0 and 3 with a mean of 0.4. Visible mould growth was recorded in only 8 or 25% of the 32 samples investigated (Table 1). When present, growth was usually in traces and this made the detection difficult. Similar observations were recorded by Adebajo (1993), on stored tiger nuts; and it was responsible for the ease with which mouldy low water activity food products, especially nuts are presented for sale after adulteration and "dressing." This practice by retailers is possible because many consumers are casual in their observation and thus unable to detect mould deteriorated samples. In the present study however, mouldy nut samples were easily detected in the mouth because they were not crispy though the pleasant aroma of the nut was still perceived and strongly too. The latter may cause children and even adults that are poorly discriminating in the choice of foods they consume to ingest mouldy samples thus potentiating a public health hazard (Adebajo, 1993).

The total fungal counts/ g obtained ranged between 180 (sample 19) and 16, 300 (sample 16) while the mean count recorded for the entire 32 samples was 3,368 (Table 1). The spread plate method adopted in this study had been found by several workers (King et al., 1986; ICMSF, 1986), to be preferable to pour plate method as it gave batter recoveries of fungi and lower dilution errors.

Specifications for fungal contamination in packaged nuts and allied foods are usually related to the nature of the product and the level of standard set by the producing company. Consequently, wide variations exist among companies and this prompted the International Commission on Microbiological Specifications for Foods (1986), to set fungal tolerances for flour, cereal and packaged nut products in the range 10^2 to 10^4/g. However, for most strict companies, acceptance standards of less than 10^3/g is adopted (King et al., 1981). The results in Table 1 therefore indicate that only 14 or 43. 8% and 28 or 87.5% of the cashew nut samples investigated were fit for human consumption at 10^3 and 10^4/g acceptable fungal levels, respectively.

Altogether, 14 fungi belonging to 5 genera were recovered at varying levels from the cashew nuts (Table 2). The most predominantly encountered species in decreasing order of isolation from the non-disinfected nuts were *Aspergillus niger, A. restrictus, A. flavus, A. fumigatus, Aspergillus,* sp*., Rhizopus nigrians, R. arrhizus* and *M. pusillus;* while *A. tamarii; Penicillium citrinum, A. ochraceus, Pencicllium* sp. While *P. digitatum* and *Syncephalastrum* sp*.* were the less frequent isolates and were not recovered from the surface-disinfected nuts.

Some of the species, especially of *Aspergillus* and *Penicillium* (Table 2) associated with the nuts are known to have strains that produce toxic metabolites (Bamburg et al., 1969; Cole and Cox, 1981). Thus, they pose a potential hazard to consumers' health. The conditions generally known to influence the production of mycotoxins in foods and allied agricultural products

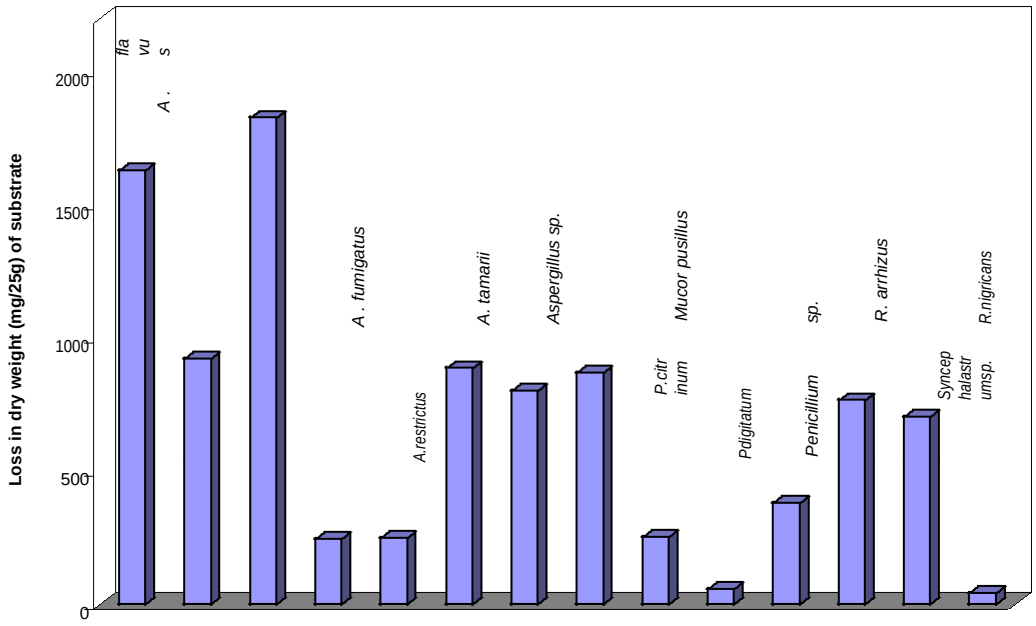

Figure 1. Fungal induced losses in dry weight of milled cashew nut after incubation at 28^{0}C for 30 days.

Table 2. Incidence[a] of fungi recovered from non disinfected (ND) and surface disinfected (SD) cashew nuts.

Fungus	ND		SD	
	MA[b]	MY[b]	MA	MY
Aspergillus flavus Link[c]	54	31	9	3
A. fumigatus Fresenius[c]	50	29	6	6
A. niger van Tieghem	66	49	17	11
A. ochraceus Wilhem[c]	17	5	1	0
A. restrictus G. Smith	59	56	8	8
A. tamarri Kita.[c]	19	9	2	1
Aspergillus sp.	51	6	4	3
Mucor pusillus Lindt	28	7	2	1
Penicillium citrinum Thom.[c]	19	5	2	0
P. digitatum Sacc.[c]	4	4	0	0
Penicillium sp.	8	3	1	1
Rihzopus arrhizus Fischer	38	14	3	2
R. nigricans Ehrenb	46	20	5	1
Syncephalastrum sp.	2	0	0	0

[a]In % of 1280 cotyledons plated (from the 32 samples).
[b]Plating medium: MA= MA40; MY= MY50G
[c]Well known producers of potent mycotoxins

include presence of a toxigenic mould, a suitable substrate for the growth of the mould, and an environment conducive for the toxin production by the mould (Betina, 1984). Thus, the high ambient temperatures (25 to 33^{0}C) and relative humidities (≥80%) prevalent in southern Nigeria (Ogundero, 1987) suggest that elaboration of mycotoxins in poorly packaged and stored retail cashew nut samples is to be expected. However, there is limited information as to the degree of the suitability of cashew nut as substrate for myctoxin production, especially aflatoxins, by *A. flavus* (Abdel-Gawad, 1993; Manay and Shadaksharaswamy, 1987). This dearth of information is worrisome because *A. flavus* was recorded in all the nut samples analysed and ranked among the three most frequently encountered species (Table 2). Of the known mycotoxins, the most important from the viewpoint of direct hazard to human health are aflatoxins (Hsieh, 1986). They pose a quadruple threat to both human and animals as they are mutagenic carcinogenic, teratogenic and toxic (Wogan et al., 1974; Chu, 1977; Pitt and Hocking, 1997). In the light of this, we have designed a series of experiments (unpublished) to determine the suitability of cashew nuts as substrate for the elaboration of aflatoxins by *A. flavus* and also to characterise the interacting effects of temperature, moisture content, water activity and time on the production of the toxins.

After the 30-day incubation period, appreciable weight reduction was recorded in each of the experimental flasks with the exception of those inoculated with *P. digitatum* and *Syncephalastum* sp. According to Eggins and Coursey (1968), the basis for biodeterioration is often the utilisation of individual compounds as nutrients by a biodeteriogen. Thus Figure 1 displays the relative abilities of the text fungi to axenically deteriorate cashew nuts under the conditions investigated. Of the six isolates known to have highly toxigenic strains (Table 2), only *P. digitatum* recorded poor growth while *A. flavus* had the largest growth thus suggesting the possibility of

formation of aflatoxins if storage conditions are conducive. This observation has further strengthened the need for close monitoring of the mycological quality of retail cashew nut samples to protect the public health.

Thus, far, the overall findings presented here show that retail cashew nut samples as packaged and sold in the Lagos metropolis are susceptible to fungal deterioration and possibly mycotoxin contamination especially during storage. Fortunately however, excellent processing and packaging techniques including protective practices that had been successfully adopted elsewhere for similar products are well documented (Desrosier and Desrosier, 1977; Frazier and Westhoff, 1978; Manay and Shadaksharaswamy, 1987; Potter 1978).

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