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Full Length Research Paper

Effect of time and relative humidity on the microbial load and physical quality of cashew nuts (*Anacardium occidentale* L) under storage

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Post harvest deterioration by microbes due to improper storage condition is considered to be the major cause of spoilage of most seeds like cashew nuts. Roasted cashew nuts were subjected to 4 different storage conditions with different relative humidity of 30, 70, 80 and 90%, respectively, for a period of 12 days. Each storage condition was examined for microbial growth, crispiness, pH changes, moisture content and other parameters. 8 fungal species identified as *Aspergillus niger, Rhizopus sp., Penicillium sp., Trichoderma sp., Botryodiplodia sp., Fusarium compactum, Aspergillus flavus* and *Aspergillus ochraceous* and 3 bacteria isolates identified as *Bacillus subtilis, Bacillus licheniformis* and *Staphylococcus* sp., were obtained. The most predominantly encountered organisms were fungal isolates of *Rhizopus sp and A. niger* with percentage frequency of 34.9 and 32.6%.

Key words: Relative humidity, preservation, microbial load.

INTRODUCTION

The cashew plant (*Anacardium occidentale* L.) is a small medium size dicotyledonous tree belonging to the order terebintals and family Anacardiacea (Tozig, 1979). A cashew fruit consists of two parts; the apple, which is really not a fruit, but a swollen penducle that grows behind the real fruit and the nut (Roth, 1974) . This large pulpy and juicy part is a pseudo fruit with a true sweet flavour and aroma. The cashew nut grows externally in its own kidney – shaped, hard shell at the end of the pseudo-fruit. The cashew nut is an achene about 3 cm long with a grey- brownish or grey green pericarp. The shell is 2 – 3 mm thick; with a leathery outer case and a thinner, harder inner case between which is a honey combs structure that contains the phenolic cashew nut shell liquid CNSL which is an excellent source of phenol for polymer production (Pillai et al., 1998) . The cashew kernel is protected from this shell liquid by a thin layer of testa.

Worldwide, cashew nuts are highly esteemed and priced food delicacy because of their pleasant taste and flavour. This growing interest has been ascribed to the purported dual roles of the kernel. It can be used as a substitute for peanut in the confectionery industries and as an important source of lipid and protein. Cashew kernel is very nutritious, it contains no harmful cholesterol, it is rich in minerals and vitamins especially thiamine which is very useful for the stimulation of appetite and nervous system. It is also very useful in anaemia, being very rich in iron. Its regular use is beneficial in the treatment of cough, urinary and liver disorders. Cashew kernel is also known to possess aphrodisiac properties

Unfortunately, during processing, cashew nut kernel being highly hygroscopic is susceptible to microbial deterioration and spoilage when not properly stored (Adebajo and Diyaolu, 2003). Being an oily seed, it has been highly implicated in human and animal pathology due to mycotoxin formation (Bacha et al., 1988). The situation may be worsened by consumers' reluctance to discard fairly mouldy cashew nut due to the irresistible taste and flavour. All earlier investigations were concentrated on the mycology of the nut without

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reference to the bacterial involved and the storage condition. This study is therefore aimed at characterizing the interacting effect of water activity, moisture content and time on the storage stability of cashew nut kernel at the prevailing environmental condition and identifying the various organisms involved.

MATERIALS AND METHODS

Collection of samples

Good grade cashew nut of the current season was obtained from the storage unit of Cocoa Research Institute of Nigeria. The nuts were carefully selected to ensure that infected ones were not included. They were transported to the laboratory in clean polyethene bags.

Removal of cashew kernel, treatment and storage

The cashew kernel was isolated using a simple cutter knife. This was used to slit each nut open and a pointed knife was employed to remove the kernel immediately from the shell to minimize contamination with the cashew nut shell liquid (CNSL). The kernel was then subjected to roasting at 80°C for 1 hour as this ensured the removal of the testa. 4 relative humidity levels of 3, 70, 80 and 90% of storage were chosen for investigation and these preferred relative humidity were created using different non-toxic desiccant inside a clean sterilized dessicator prepared according to the method of Bates and Winston (1960).

Weighted amounts (120 g) of the roasted cashew kernel were aseptically placed on sterile Petri-dishes with perforated holes in each of the desiccators, sealed with vaseline to ensure they were air tight and incubated at room temperature (27 $\pm$ 2°C). Samples were taken after every 3 days from each of the storage for analysis for a period of 12 days (3, 6, 9, and 12 days).

Moisture content and pH analyses

Moisture content was determined according to the recommended British Standards Institute method (BS 4289, part 3, 1968) for the determination of moisture in oil seeds (S.W. Pixton 1982). 10 g of the kernel were taken from the 4 desiccators after every 3 days and were subjected to drying at 103°C for 3 h and then for further period of 1 h until a constant weight was achieved. The pH values were obtained by pulverizing the samples in a sterilized mortar. Suspensions of the pulverized material in distilled, de-ionized water was obtained and used to determine the pH values.

Isolation of microorganisms from the samples

Considering the design of the experimental set up of 0, 3, 6, 9 and 12 days at different relative humidity of storage, samples were randomly taken from the different desiccators, weighed and surface sterilized with 0.1% of mercury chloride solution (HCl_2) for about 2 min. This was followed by rinsing with 3 washes of sterile distilled water. 10 g of the nut was pulverized by grinding with mortar and pestle that has been sterilized in an autoclave. The initial dilution was prepared by adding 10 g of the pulverized sample to 90 ml of distilled water. Decimal dilutions (1 + 9 ml) were prepared down to 10^{-3} using the serial dilution method of Meynell and Meynell (1970). Using sterile pipettes, 1 ml of 10^{-1} and 10^{-2} dilution of the various samples was plated out by mixing with sterilized agar using the pour plate method (Harrigan and MacCance, 1974). Before pouring plates, chloramphenicol was added at a concentration of 50 mg/L to potato dextrose agar (PDA) to inhibit the growth of bacteria. Also, nystatin was equally added at a concentration of 50 ml/L to nutrient agar to inhibit the growth of fungi. The plates were swirled round for the distribution of the inoculum and setting of the agar. After solidification, the Petri-dishes were incubated upright at room temperature for 24 – 48 hours, respectively. The colonies observed were counted and sub-cultured for identification.

Identification of isolates

All the isolates were identified by microscopic, biochemical and physiological characteristics and by reference to identification manuals. Subsequently, occurrence of different fungi were grouped and used for the assessment of the percentage of frequency.

Effect of different storage conditions on the physical state of the samples

At different time intervals (0, 3, 6, 9 and 12 days) samples of the kernel were picked from each of the desiccators to determine the level of crispness. This was done by bending the kernel in between the finger. The crispy ones broke easily while the non-crispy ones were wet.

RESULTS

8 fungal isolates were obtained from the various treatments of the cashew nuts. They were identified as *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Botryodiplodia* sp., *Rhizopus* sp., *Fusarium compactum, Trichoderma* sp. and *A. ochraeous* while the bacteria isolates were identified as *Bacillius subtilis, Bacillius licheniformis* and *Staphylococcus* sp., respectively. The different fungal isolates obtained at different relative humidity of storage with respect to time are shown in Table 1. The succession of growth of the various fungal isolates in the various treatments differed as some fungi were eliminated by the treatment. However, 2 fungi isolates *Rhizopus sp* and *A. niger* were predominantly encountered in all treatment but their number were greatly affected as depicted in the fungal count as shown in Table 2.

At relative humidity of 90%, there was an increase in the number of fungal count on the 12th day of storage. Highest counts were observed in similar observation but reduced numbers of counts were noted in storage at 70, 80 and 30% relative humidity, the lowest number of counts were observed. The most predominant encountered species and the frequency of occurrence in decreasing order of isolation from the different storage condition are shown in Table 3. Few bacteria growth was observed in the study (Table 4). In some treatments, the bacteria growth observed was in traces especially at 30% relative humidity while in some, growth was not even observed at all.

The moisture content (%) recorded for the samples at the various treatment ranged between (4.0 - 12.0) as shown in Table 5. Relative humidity of the cashew nut

Table 1. Fungal isolates in cashew kernel stored at different relative humidity and time.

Time	Relative humidity (%)/fungal isolate			
(Days)	30	70	80	90
0	No growth	No growth	No growth	No growth
3	*Rhizopus sp.* *A. niger* *Botryodiplodia sp.*	*Rhizopus sp.* *Penicillium sp.* *A. niger*	*Rhizopus sp.* *A. niger* *Penicillium sp.*	*Rhizopus sp.* *A. niger* *Penicillium sp.*
6.	*Rhizopus* *A. niger*	*Penicillium sp.* *A. niger* *Trichoderma sp.* *F. compactum*	*Rhizopus sp.* *A. niger* *A. ochraceous*	*Rhizopus sp.* *A. niger* *Penicillium sp* *F. compactum.*
9	*Rhizopus sp.* *A. niger*	*A. flavus* *Rhizopus sp.* *Trichoderma sp.*	*Rhizopus sp.* *A. niger*	*Rhizopus sp.* *A. niger* *A. flavus*
12.	*Rhizopus sp.*	*Rhizopus sp.* *A. niger*	*Rhizopus* *A. niger*	*A. flavus* *Rhizopus sp.* *A, niger*

Table 2. Fungal counts in cashew kernel exposed to different relative humidity at different time intervals.

Time (Days)	Relative humidity (%) /fungi counts (cfu/g)			
	30	70	80	90
0	-	-	-	-
3	6	9	10	57
6	3	13	63	85
9	5	19	75	122
12	4	48	101	140

- = No growth.

Table 3. Frequency of occurrence of different fungal isolates in cashew kernel exposed to different relative humidity at different time intervals.

Fungi isolate	Frequency of occurrence (%)
Rhizopus sp.	34.9
A. niger	32.6
Penicillium sp.	11.6
A. flavus	4.6
Trichoderma sp.	7.0
Fusarium compactum	4.6
A. ochraceous	2.3
Botryodiplodia sp.	2.3

before storage was 4.0. This value was maintained throughout the course of investigation at relative humidity of 30%. The moisture content at relative humidity of 70, 80 and 90% increased with time. At each of the different relative humidity, the moisture content was observed to display a gradual increase with increase in days of storage. For all, the peak was reached on the 12th day. The presentation in Table 6 shows the mean pH values recorded for the samples in the setup at different time intervals. At relative humidity of 30%, a gradual increase in pH was observed with increase in days of exposure. It increased from pH 6.35 on day 3 to 6.92 on day 6 only to drop to 6.69 on the 12th day. However, at relative humidity of 90%, it increased from 6.35 on the 3rd day to a maximum of 6.83 on the 6th day and dropped to a minimum of 5.07 on the 12th day.

The influence of the different relative humiditiy on the physical state of cashew nut at the different time intervals was equally evaluated as indicated in Table 7. Treatment at 30% relative humidity showed the kernels to be very hard and crispy. With 70, 80 and 90% relative humidity, the nuts were a bit crispy on the 3rd day of examination and these decreased with time and on the 9th and 12th day the nuts stored at 90% had already grown mouldy.

DISCUSSION

Fungi were the predominant organisms isolated from the cashew nut kernel with few bacteria isolates at different relative humidity of storage. Some groups of these fungi have been reported by Adebajo and Diyaolu (2003) as the most predominantly encountered species of fungi from deteriorating cashew nuts. Some of the species, especially *Aspergillus* and *Penicillum* associated with nut are known to have strain that can produce toxic metabolites (Bamburg et al., 1969; Cole and Cox, 1981)

Table 4. Bacteria counts in cashew kernel exposed to different relative humidity at different time intervals.

Time (Days)	Relative humidity (%)/ bacterial counts x 10^{-1} (cfu/ml)			
	30	70	80	90
0	NG	NG	NG	NG
3	5	10	NG	20
6	NG	NG	22	NG
9	NG	8	5	19
12	3	15	16	NG

NG = No growth.

Table 5. Moisture content of cashew kernels obtained after storage at different relative humidity for 12 Days.

Time (Days	Relative humidity (%)/ moisture content (%)			
	30	70	80	90
0	4.0	4.0	4.0	4.0
3	3.0	5.0	5.0	8.0
6	2.0	6.0	8.0	9.0
9	4.0	9.0	8.0	9.0
12	4.0	9.0	9.0	12.0

Table 6. The pH changes in suspensions of cashew nut stored at different relative humidity for 12 days.

Time (Days)	Relative humidity (%) /pH			
	30	70	80	90
0	6.1	6.1	6.1	6.1
3	6.35	6.30	6.32	6.35
6	6.92	6.75	6.77	6.83
9	5.94	5.95	6.07	6.04
12	6.69	6.85	6.73	5.07

Table 7. Effect of different relative humidity on the physical state of cashew kernel stored for 12 days.

Time	Relative humidity (%)/physical state			
(Days)	30	70	80	90
0	+++++	+++++	+++++	+++++
3	+++++	++++	++++	+++
6	+++++	+++	+++	++
9	+++++	++	++	+
12	+++++	++	++	+

+++++ = Very Crispy and hard; ++++ = a bit crispy; +++ = not crispy; ++ = wet; and + = mouldy.

and can survive in a wide range of environment (Pitt, 2000).Thus, they pose a potential hazard to consumers' health even at low level (Paranayama et al., 2003). The condition generally known to influence the production of mycotoxin in food and allied agricultural products include the 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), the latter of which formed the crux of the present investigation.

The interacting effect of relative humidity on the growth of the fungi shows that 30% relative humidity was the best storage condition as some fungi were eliminated and even where growth occurred, it was in traces that decreased with time. This finding is in agreement with Brocks (1984) that when an organism grows in a medium with low water activity due to the addition of solute, the organism must perform work to extract water from the solution. This generally results to reduced growth yield or lower growth rate. The cytoplasm of microorganisms usually has a higher solute concentration, so water tends to flow into the cell, but if the external solute concentration is raised to a value higher than the internal, water will flow out and an organism can only obtain water and grow under this condition by increasing its internal solute concentration which is an energy requiring process.

However, at higher relative humidity of 70, 80 and 90%, there were different succession of organism with time. Increase in time of storage has been known to also lead to increase in fungal count. Adebajo and Diyaolu (2003) observed that relative humidity of 70% is generally considered as the maximum level for safe storage of cashew nuts (Pixton, 1982; Henderson, 1985) and that relative humidity ($\geq$ 80%) prevalent in Southern Nigeria (Ogundero, 1987) suggests that spoilage and mycotoxin formation in cashew nut is to be expected. The most frequently encountered fungi species with the highest frequency of occurrence were *Rhizopus* (34.9%) and *A. niger* (32.6%). This conforms with the findings of Adam and Moss (1995) that the most important lipolytic mould are species of *A. niger, A. tamari* and *Penicillium sp.* while at higher water activities, species of *Rhizopus* may be implicated. Also, Salunkhe and Desai (1986) observed that storage mould especially *Aspergillus* group develops quickly under high humid conditions, increasing the fatty acid content of oil seeds.

For the bacterial isolates, few were encountered and in some treatment, growth was not even observed. This finding could probably be due to the fact that cashew nut being a slightly acidic product in terms of the inner content does not support the growth of bacteria. The circumstantial evidence for the involvement of bacteria in the deterioration of stored cashew nuts is the ability of the spores of bacillus to resist desiccation and this allows their survival in dried products (Adams and Moss, 1995). The lipolytic activities of *Bacillus sp.* are well known; they have been isolated from oily foods. From the studies conducted by Jonsson and Snygg (1974), *B. licheniformis* was one of the organisms identified with the greatest ability to hydrolyse the fat in mayonnaise and liver paste. Also, Odunfa (1989) observed *B. subtilis* to be strongly lipolytic on palm oil.

Further evidence in support of this is the fact that insufficient heating which will destroy vegetative cells, may not destroy spores of *B. subtilis,* some of which are known to be resistant to 100°C for 15 - 20 min (Atlas and Bartha, 1981) and may even withstand higher temperatures in a protective medium such as oil and cashew nut. The moisture content (%) recorded for the cashew nuts during the different storage conditions ranged between 4.0 and 12.0. Storage at 30% relative humidity throughout the period of time showed that the moisture content was relatively constant at 4.0 hence; the nuts were hard, crispy and were not predisposed to microbial spoilage.

Relative humidity of 70, 80 and 90% showed an increase in moisture content with time and with peak value of 12.0% observed after 12 days of storage at 90% of relative humidity when the nuts had already grown mouldy. This shows that cashew nut being colloid, are hygroscopic absorbing moisture from the surrounding atmosphere hence, the increase in moisture content above the storage limit was observed and consequently the nut were predisposed to mouldy growth. This is in agreement with the finding of Pixton (1967) who observed that agricultural products including cashew nut would absorb moisture from the surrounding atmosphere until they are in equilibrium with it.

The pH values obtained shows that the cashew nut is a low acid food product and without exception all the samples had pH value conducive for microbial growth and activities. Relative humidity of 90% on the 12th day of storage showed a significant decrease in pH values from 5.07, which could be responsible for the increase in fungal growth that was encountered. Similar observation was made by Frazier and Westhoff (1978) and Smith and Moss (1985). Thus, as far, the overall findings presented here shows that cashew nut kernel is highly hygroscopic and therefore there is need for proper storage and close monitoring of the microbiological quality in order to protect public health. Furthermore, the result had equally shown that the shelf life of cashew nut can be extended by the modified atmospheric storage in line with the work of Booth (1984) that the post harvest loses of oil seeds can be minimized by controlling the environmental factors in such a way that activities responsible for deteriorative changes are reduced to a minimum.

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