Mycoflora Associated with Post Harvest Processing Stages of Kolanut (Cola Nitida Vent Schott of Endlicher)

S.O. Agbeniyi, Otuonye, H.A. and A. R. Adedeji Cocoa Research Institute of Nigeria

Abstract

The mycoflora associated with processing stages of kolanut post-harvest were evaluated at the Cocoa Research Institute of Nigeria, Ibadan Nigeria. Several samples of healthy and infected kolanut were obtained at various stages of primary processing (skinning, washing, sweating and storage). Fungi isolations were carried out by plating each sample of visibly healthy and infected nuts on malt extract agar in 9-cm petri dishes at 25° C. Similarly frequency of occurrence of fungal isolates were evaluated from the nut and the processing water. Botrydiplodia Theobramae, Fusarium pallidoroseum, Aspergillus Sp. Penicillum sp. Curvularia sp. and Mucor sp. were isolated from the infected kola nuts. The frequency of occurrence of B. theobromae and F Pallidoroseum was 0.7 and 0.6 respectively in kolanuts obtained after the washing stage. whereas, it was 0.12 and 0.37 respectively in nuts obtained during storage. The mycoflora of the kolanut after the washing stage were not markedly different from that obtained during sweating (P = 0.05). The diameter of colonies of the most common fungi encountered were also determined. The mycelial growth of F. Pallidoroseum ranked highest after five days of incubation.

Introduction

Cola nitida (vent) Schott and Endlicher is the most widely cultivated Cola species and Cola of commerce in West and Central Africa. The bulk of the Kolanut produced in Nigeria which is the world largest producer. is consumed as fresh nut. The habit, if not comparable to the smoking of tobacco of Western civilization on, or opium usage of Far Eastern societies, certainly show similarity to the Betel nut chewing of the Asiatic community (Agatha *et al.* 1978). The habit of chewing the odourless and astringent tasting nut has enhanced its continued use for about 1000 years in West Africa. The dried sliced nut is a commercial export commodity for the preparation of beverages and soft drinks (Olunioyo, 1979). The presence of alkaloids and other chemicals in kolanuts also makes them suitable for drug preparations.

The susceptibility of kolanuts to fungi infection has been reported (Odebode, 1990.). However, studies - to -date have not elucidated the mycoflora associated with each processing stage of kolanuts. At present, there are no chemicals recommended for reducing the nut - borne fungi of Kolanut. Infected nuts decay rapidly and though there is little secondary spread, the surrounding nuts are covered with masses of fungal spores. This contamination of healthy nuts with spores from rotted nuts is often a greater economic problem in fresh market producing areas. Kola traders often control spoilage by removing diseased nuts at intervals during storage, therefore, creating a need for intensive research on alternative options for disease control. Consequently, this study was therefore designed to determine the mycoflora associated with kolanuts at each processing stage and to evaluate the frequency of occurrence of the fungal isolates. This is with a view to optimize control measures.

Materials and Methods

Usual post-harvest handling practices for fresh kolanut grown at the Cocoa Research Institute of Nigeria were evaluated for incidence fungi.

The Processing Water

The microbial flora of the processing water obtained from River Oda-Ona and run through pipe to the processing unit were determined. One ml of the water sample used during skinning and washing stage were plated out on malt extract agar acidified with 70% lactic acid to eliminate bacteria in 9-cm petri dishes. Similarly, samples of kolanut were soaked in sterile distilled water and rinsed also in sterile distilled water. One ml of the water sample (sterile distilled water) used warm plated out on malt extract agar for fungal isolation.

The Skinning Stage

The microbial flora of the kolanuts obtained at skinning stage were determined. Ten kolanuts samples were obtained randomly and cultured either on wet 11cm Whatman Filter paper (Grade 41 ashless, Kent, England) in 11cm diameter petri dishes or cut in several piece of 4mm and plated directly on malt extract agar as described above. The plates were incubated at 25°C for 15 days and fungal colony that developed were recorded at 24 hrs interval. The frequency of occurrence of each fungal genus was calculated as described by Britton *et al,* (1993.)

The Sweating Stage

The mycoflora of the kolanuts obtained at sweating stage were determined. The nuts were subjected to 5 days curing process prior to storage at 25° C. Ten samples of Kolanut were cultured daily for the whole period of sweating in malt extract agar as described above. The plates were incubated at 25° C for 10 days. The frequency of occurrence of fungal genus was determined as stated above.

Table 1. mycoflora of processing stages of kolanut

Fungal isolates	River water	Sterile water	Skinning		Sweating			Storage
			Soaked with river water	Kolanut soaked with sterile water	D1	D2	D3	
B. theobromae	-	-	+	+	+	+	-	+
F. pallidoroseum	-	-	+	+	-	+	+	+
Aspergillus sp.	-	-	+	+	+	+	+	+
Penicillium sp	+	-	+	+	+	+	+	-
Mucor sp	+	-	+	-	-	+	+	-
Paecilomyces variotii	-	-	-	+	+	-	+	+

+ = Present
- = absent
D1 = Day 1, D2= day 2, D3= day 3.

Table 2. Frequency of occurrence of fungal isolates

Fungal isolates	River water	Sterile water	Skinning		Sweating			Storage
			Soaked with river water	Kolanut soaked with sterile water	D1	D2	D3	
B. theobromae	0.00	0.00	0.50	0.37	0.12	0.25	0.12	0.12
F. Pallidoroseum	0.00	0.00	0.40	0.25	0.25	0.10	0.25	0.37
Aspergillus sp.	0.20	0.00	0.30	0.25	0.37	0.10	0.12	0.37
Penicillium sp	0.18	0.00	0.10	0.25	0.12	0.10	0.12	0.00
Mucor sp	0.17	0.00	0.10	0.00	0.12	0.00	0.12	0.00
Paecilomyces variotii	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.10

D1= day one
D2 = day two
D3 = day three

The Storage Stage

The microbial flora of the kolanuts obtained after five weeks of storage were determined. Samples of kolanut as described above were obtained randomly and cultured in malt extracts agar at 25° C. The frequency of occurrence of fungal genus was calculated as stated above.

Diameter of Colonies of Fungi isolated from kolanuts

The diameter of colonies of the most common fungi encountered were determined. Each fungus was maintained on potato dextrose agar (PDA) for five days at 25°C. Five replicate 8mm diameter agear discs were removed from the edge of an actively growing culture of each fungus and placed in 9 cm diameter plastic petri dishes containing 20 ml of PDA amended with 70% lactic acid to suppress bacteria. Five replicate plate of each fungus was incubated at 25°C and radial growth of mycelia was measured along two perpendicular axes. The mean of the two measurements was calculated.

Results and Discussion

The mycoflora of the river water used to process fresh kolanuts include Aspergillus fumigatus, A. niger, Penicillium sp. and Mucor sp. (Table 1). Thus in the absence of other inoculum from the field and during storage, kolanut processed from the river water could suffer significant deterioration during storage. Consequently, appropriate sanitation procedure of the river water which aims to produce nuts that is safe for the consumer and of good storage quality is suggested.

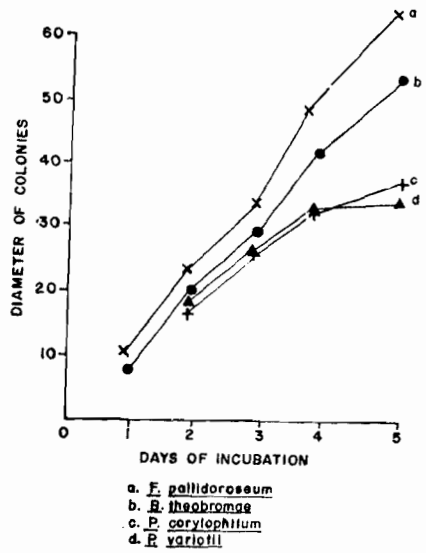
When freshly harvested kolanuts were soaked in the processing water,, additional fungi were isolated (Table 1). This include Botrydiplodia theobromae, Fusarium Pallidoroseum and Paecilomyces variotii. The isolations of these fungi free nuts suggest that they were nut-borne fungi from the field. Similarly, appropriate sanitation procedure designed to reduce microbial load of the harvested kolanut is recommended. The results of this investigation clearly revealed the sources of inoculum responsible for the deterioration of kolanuts. Thus washing of kolanuts with chemicals generally recognized as safe will help in minimizing fungal infection during storage. Furthermore, the high moisture content of 54-64% (Ogutuga, 1975) in kolanut enhances its susceptibility to fungal infections. Subsequently, curing of kolanut at 30 C for 48 hours was recommended prior to storage (Agbeniyi & Fawole, 1999).

There were sharp differences in the frequency of occurrence of fungal isolates in each stage of primary processing. For instance, the frequency of occurrence of B. theobromae during

skinning was 0.37 whereas it was 0.00 in the processing water (Table 2). Similarly, the frequency of occurrence of Mucor sp. was 0.17 during skinning but was 0.05 during storage. However, no marked difference in the frequency of occurrence of Peneicillium sp. and Paecilomyces variotii during skinning and sweating (Table 2). From the results presented in Table 1, the microbial population increase during soaking, skinning and sweating processes. It is evident that F. pallidoroseum and B. theobromae were most frequently encountered. Subsequently, meaningful control trials should begin at the onset of primary processing procedures. The scarcity of previously published reports on the occurrence of storage rot on kolanuts suggests that the microbial contamination is sporadic or non-existent in many fresh market production areas.

Furthermore, the high frequency of B. theobromae during the primary stages was not surprising, considering the fact that both nuts obtained from farm litter and on-tree were processed together. The study indicates that kolanuts were naturally infected during harvesting and disseminated along the processing stages. To better understand the interaction of the mycoflora found in kolanuts, *in vitro* studies were conducted. Both the Fussarium and Botryodiplodia showed growth 24 hrs after incubation (Fig.1). This trend was maintained throughout the five days of incubation. The investigation clearly indicates the ability of the Fusarium and Botryodiplodia to establish faster on kolanut than the other organism (Fig.1).

Figure 1: Diameter of colonies of Fungi associated with kolanuts i culture.



Conclusions

The results from the present study clearly indicate that kolanut are susceptible to fungal infection. The occurrence of fungal isolates is accentuated by the adherence of spores to the nuts, even after washing, which is then introduced and disseminated in various stages of processing. Hence, appropriate sanitary procedures which is aimed at controlling the growth of disease-causing organisms or the production of toxins is recommended.

Acknowledgement

The authors are very grateful to the Director & Chief Executive of Coca Research Institute of Nigeria for permission to publish this work. We also acknowledge the contributions of Mrs. C. O. Ogundipe and Mrs. C A Aboderin.

References

Agatha M, Breckenldge, C and Soyemi, E. A (1978). Some preliminary observation on the effects of kolanuts on the cardiovascular system. Nigeria Medical Journal 8 (6) 501-505.

Agbeniyi, S. O. and Falowe, B. (1997). Effects of curing and pre-storage die treatments on stograge mould of kolanuts. European Journal of Food Research & Technology 208: 47-49.

Britton, K. O. Roncadori, R. W. and Hendrix , F.F (1993). isolation of Discula destructiva and other fungi from seeds of dogwood trees, Plant Dis. 77: 1026-1028.

Odebode, A. C. (1990): Post Harveest rot of kolanuts caused by Botryodiplodia therobromae and Fusarium Pallidoroseum. Ph. D Thesis Univ., Ibadan.

Oguntuga, D. B. A. (1975): Chemical composition and potential commercial uses of kolanuts (cola nitida). Ghana Journal Agric. sc. 8: 121-125.

Olunloyo, o. A. (1979): Fungi associated with deterioration of stored kolanuts. Nigerian Journal of Agric. Sc. 1(1) 151-159.