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Full Length Research Paper

Fungi Associated with the Spoilage of Pineapple Fruits in Eke Awka Market Anambra State.

* Onuorah, S.C.; Udemezue, O.I; Uche, J.C and Okoli, I.C.
Department of Applied Microbiology & Brewing, Nnamdi Azikiwe University, Awka, Anambra State, * Corresponding Author E-mail: Sonuorah484@yahoo.com.

ABSTRACT

The fungi associated with the spoilage of pineapple fruits in Eke Awka, Anambra States were studied. Spoilt and healthy pineapple fruits were used in the study with sabouraud dextrose agar as the culture medium while the pour plate technique was employed in the fungal isolation. Pathogenicity test was also carried out using healthy pineapple fruits. The fungi were isolated, and identified on the basis of their colonial and morphological features as the species of Aspergillus, Fusarium, Rhizopus, Penicillium and Candida. The fungal counts ranged between $1.0x10^6$ cfu/g and $3.1x10^6$ cfu/g. Samples 2 had the lowest count of $1.0x10^6$ cfu/g. Aspergillus sp occurred in all the samples studied while fusarium sp had the least occurrence, having been isolated from sample 1 only. The result of the pathogenicity test revealed that all the species of fungi isolated from the spoilt pineapple fruits were also reisolated from the healthy fruits into which they were inoculated, indicating that the organisms were responsible for the spoilage of the fruits.

Key words: Fungi, Spoilage, Pineapple fruits, Eke Awka Market, Anambra State

INTRODUCTION

Pineapple (Ananas Comosus) is a vegetatively propagated fruit crop. It is one of the few crops in which cultivars are derived from spontaneous mutations and natural evolution without controlled breeding (Osei-kofi et al, 1997).

Pineapple is a cylindrical false fruit (pseudo-fruit) of the family Bromeliaceae and consists of a thickened, fleshly, very juicy axis core and inedible, scaly, warty skin, resembling a pine core. Only the polygonal, flattened outsides of the individual fruits are visible at the surface of the multiple fruit (syncarp). The fruit is topped by a crown of prickly leaves. The axis core (central cylinder) in the middle of the false fruit is woody and therefore inedible.

Important commercial varieties include "smooth Cayenne", the most important variety in the Canning industry; the yellow "Queen", which is cultivated for eating fresh and the Spanish group "Red Spanish", likewise suitable for eating fresh.

Pineapples contain high level of sugars and other nutrients and their low P^H values make them particularly desirable to fungal decay. Fungi can survive and grow on pineapples and their nutrients such as carbohydrates, proteins and fats support the growth of pathogens.

Ripening pineapple fruit is susceptible to infection by a variety of disease-causing

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microorganisms including fungi. These disease tend to develop and damage the fruit during fruit nutrition, starting from twenty days before the fruits are harvested until they reach the consumer as fresh fruits or are processed in cannery as canned pineapple, thus the internal quality of the fresh fruit is reduced significantly due to attack by a complex of microorganisms such as penicillium, fusarium and Yeasts which are believed to cause black spots of pineapple fruits.

In developing contries such as Nigeria, post harvest losses are often more severe due to inadequate storage and transportation facilities (Droby, 2006; Khali and Mazher, 1994). Pineapple infection may occur during the growing season, harvesting, handling, transport, post harvest storage and marketing conditions or after purchasing by the consumer. Another major source of contamination is the washing water (Khali and Mazher, 1994).

The process of infection follows the development of fungal penetrating structure called appresorium. The colonization process by fungi involves their ability to establish themselves within the host. This is initiated when the fungi following adhesion and release of enzymes depolymerize certain cell wall polymers such as prospection, the cementing substance of the produce.

The primary cell wall of pineapples is composed of approximately 10% proteins and 90% polysaccharides which can be divided into three groups: cellulose, henicellulose and pectin (Nathalie, 2006). Numerous cell wall degrading enzymes can be secreted by fungi to breach and use the plant cell walls as nutrient sources. These fungi produce an abundance of extracellular pectinases and hemicellulases that are important factor in their spoilage of pineapples (Nathalie, 2006) leading to reduced post harvest life and the development of undesirable quality and soft rot (Miedes and Lorences, 2004). In addition, many fungal species are capable of producing mycotoxins, which are secondary metabolities that are highly toxic to humans and animals. (Al-Hindi et al, 2011).

The consumption of pineapples has been on the increase in Nigeria. This is so because they are easily accessible, nutritious and relatively cheap (Nwachukwu et al, 2008). The increase in consumption has been linked with a parallel incease in food-borne illnesses (Mensah et al, 2002). Pineapple fruits are processed and sold by unlicensed street vendors with poor education and lack of training in food hygiene (Barro et al, 2006). These fruits are usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further fungal infections.

The objective of this work is therefore to isolate, characterize and identify the fungi associated with the spoilage of pineapple fruits in Eke Awka market, Anambra State, Nigeria .

MATERIALS AND METHODS

Samples collection and processing

Twelve pineapple fruits (four spoilt and eight healthy) were purchased from different locations at Eke Awka market, Anambra State and conveyed to the microbiology laboratory in different sterile polythene bags. One gram of each of the spoilt pineapple fruits was carefully cut with a sterile scalpel and enriched in sterile Sabouraud dextrose broth (SDB) for twenty four hours. Ten fold serial dilutions were thereafter prepared.

Fungal Counts

The pour plate method was used. Aliquots of the serially-diluted samples (10^7) were introduced into sterile culture

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plates. Sterile Sabouraud dextrose agar (SDA) was introduced into the plates containing chloramphenicol at a concentration of 0.05mg/l to inhibit bacterial growth. Incubation was carried out in an inverted position for five days at room temperature and the colonies that developed after incubation were counted, subcultured on sterile SDA plates and later stored on sterile SDA Slants for identification.

Identification of the Isolates

The isolates were identified on the basis of their cultural and morphological features. The microscopic examination was carried out using the lacto phenol cotton blue solution. A drop of the solution was placed on a clean slide and a fragment of the test fungus teased out and introduced into the stain. The fungus was properly spread on the slide with the aid of a sterile needle. A cover ship was gently placed on the slide to eliminate air bubbles. The slide was thereafter mounted and examined under the microscope. Identification followed the descriptions of Chukwura *et al* (2010).

Pathogenicity Test

This was carried out using the method of Chukwura et al (2010). Four healthy pineapple fruits were washed with tap water, rinsed with distilled water and surface sterilized with 75% ethanol. Holes were made in each of the fruits with a sterile 4mm Cork borer. Each of the isolated fungi was used to inoculate the fruits and the cores of the fruits replaced.

The holes were sealed with petroleum jelly to prevent contamination. Controls consisted of four fruits wounded with the cork borer but not inoculated. The inoculated fruits and controls were placed in clean polythene bags (one fruit per bags), each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at room temperature for five days after which the fungi were re-isolated and compared with the original isolates.

RESULTS AND DISCUSSION

Studies on the fungi implicated in the spoilage of pineapple fruits in Eke Awka market, Anambra State showed that the fruits contained a teeming population of fungi. The fungal counts ranged from $1.0x10^6$ cfu/g to $3.1x10^6$ cfu/g. Sample 2 had the highest count of $3.1x10^6$ cfu/g while sample 4 had the least count of $1.0x10^6$ cfu/g (Table 1).

Table 1: Fungal counts of the spoilt pineapple fruits

Samples	**Fungal Count ($X10^6$ cfu/g)**
1.	1.1
2.	3.1
3.	2.5
4.	1.0

The fungi were identified as Aspergillus sp, Rhizopus sp, penicillium sp, fusarium sp and Candida sp (Table 2). This result is in agreement with the report of Jolaosho et al (2010) who isolated Rhizopus stolonifer from sliced packaged pineapple samples in Ogun state. Splittstoesser (1989) implicated fungi as contaminants of fresh fruits such as pineapples especially in the presence of injuries like slicing. Akinmusire (2011) also isolated Aspergillus flavus and Candida tropicalis from pineapple samples in Maiduguri, North east Nigeria. Effiuvwevwere and Oyelade (2000) also reported that Aspergillus sp and Candida sp are responsible for the rottening of pineapple fruits.

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Table 2: Colonial and Morphological features of the fungi isolated from the spoilt Pineapple fruits.

Isolates	Colonial Features	Morphological Features	Organism
1	Black Colonies with white edges	Conidia heads are large, globose,dark brown and biseriate. Conidia are globose and rough walled. Conidiophores are smooth walled	Aspergillus sp
2	Whitish colonies, growing rapidly and filling the petridish with dense cottony mycelium and becoming brownish-black with age .	Non-septate mycelia. Sporangiophores are smooth walled. Sporangia and columella are subglobose. Sporangiospores are ovoid in shape.	Rhizopus sp
3	Green and velvety	Colonies are smooth and ellipsoidal Conidiophores are smooth and short. Mycelia are arranged irregularly with branches of various lengths.	Penicillium sp
4	Pink and cottony colonies	Microcondia are ovoid in shape. Macroenidia are borne on phialides on branched conidiophores. Septate fusiform, slightly curved and pointed at both ends is present.	Fusarium sp
5	Creamish, smooth, convex and opaque colonies with a yeasty odour	Budding, spherical to elongated cells, forming pseudomycelium	Candida sp

Aspergillus sp had the highest occurrence in the samples examined, followed by Rhizopus sp and Candida sp while fusarium sp occurred less frequently (Table 3). This result agreed with the report of Akinmusire (2011).

Table 3: Occurrence of the fungi in the spoilt pineapple fruits

Samples	Aspergillus sp	Rhizopus sp	Penicillium sp	Fusarium sp	Candida sp
1	+	+	+	+	-
2	+	-	-	-	+
3	+	+	+	-	+
4	+	+	-	-	+

+ = detected
- = not detected

The pathogenicity test result (Table 4) showed that the fungi isolated from the healthy pineapple fruits had the same colonial and morphological features as the ones inoculated into them from the spoilt samples, indicating that they were responsible for their spoilage.

Table 4: Result of the Pathogenicity Test On The Healthy Pineapple Fruits

Samples	Aspergillus sp	Rhizopus sp	Penicillium sp	Fusarium sp	Candida sp
1	+	+	+	+	-
2	+	-	-	-	+
3	+	+	+	-	+
4	+	+	-	-	+

+ = detected
- = not detected

The presence of these fungi in pineapple fruits is a health risk to the consumer. Their presence can be linked to a number of factors such as improper handling and processing, use of contaminated water during washing, cross contamination from other fruits during their transportation, use of dirty processing utensils such as knives and trays, physiological and physical conditions of the produce and extrinsic parameters to which they were subjected..

Colonization of pineapple fruits by microorganisms involves their ability to establish themselves within the produce and is initiated when they degrade certain specific cell well polymers such as the protopectin which is the cementing substance of the produce. Pineapple fruits are highly susceptible to microbial spoilage but there is a variation in the susceptibility which is due largely to the differential chemical composition such as P^H and moisture content, thus the low P^H and high

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moisture content of the fruits make them more prone to fungal spoilage.

Generally, spoilage fungi are considered toxigenic or pathogenic. The fungi isolated in this study have been reported to produce secondary metabolites in plant tissues. These secondary metabolites are potentially harmful to humans and animals. Pathogenic fungi, on the other hand, could cause infections or allergies. Aspergillus sp is known to produce produce ochratoxins, a mycotoxin which is a very important toxin worldwide because of the hazard it poses to human and animal health, thus extra care should be taken during the harvesting, cleaning, sorting, packaging, transportation and storage of pineapple fruits. The control of spoilage of pineapple fruits by fungi is inevitable, therefore adequate hygiene by pineapple handlers should be encouraged as it would reduce the incidence of such organisms on the fruits as well as the health risk posed to the consuming public.

CONCLUSION

The high occurrence of fungi on the pineapple fruits demands adequate microbiological knowledge and hygienic handling practices of the fruits. These would help minimize wastes due to deterioration and unacceptability. Although the high moisture content of pineapple fruits is a serious limiting factor in their preservation, handling methods that preserve the fresh harvest quality of the produce may minimize the development of decay. An appropriate sanitation programme must be implemented so as to protect the fruits from attack by these fungi.

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