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ISOLATION AND IDENTIFICATION OF MICROORGANISMS INVOLVED IN SPOILAGE OF FRUITS OBTAINED FROM MARKETS IN KANO METROPOLIS.

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Abstract

The microorganisms associated with the spoilage of fruits purchased fresh fruits (orange, water melon, banana, apple and pineapple) from various markets in Kano metropolitan were investigated. The pour plate method was used for the isolation. A total of nine species of microorganisms were isolated and identified in the microbial examination of spoilt fruit study. They comprise of seven bacterial and three fungal species. The seven bacterial species were Bacillus, Enterobacter, citrobacter, Acinobacter, Klesbsiella, Acromonas (Aeromonas) and Micrococcus Sp. Bacillus Sp and Micrococus Sp occurred in samples obtained from all Klebsiella Sp, was isolated only from two samples while Acinobacter Sp was isolated only from one sample.(orange and banana) The three fungi isolates were mucorSp, Saccharomyces Sp and Geotrichum Sp where asGeotrichum Sp and Saccharomyces Sp occurred in samples (orange, water melon, banana, apple and pineapple) obtained from Yankaba and Yanlemo markets. MucorSp was present only in two samples bought. The bacterial counts range was 4.8 to 6.1 u 10^4cfu/g. while the fungal count range was 2.1 – 4.8 x 10^4cfu/g proper handing methods of fruits to ensure food safety were discussed.

KEY WORDS: microbial, fruits, disease, spoilage, preservation.

INTRODUCTION

The fruits are part of flowering plant that derives from tissues of the flower, one or more ovaries, and in some cases accessory tissues. Many of them that bear edible fruits, in particular, have propagated with the movements of humans and animals in a symbiotic relationship as a means of seed dispersal and nutrition, respectively.In fact, humans and many animals depend on fruits as a source of food. Fruits accounts for a substantial fraction of the world's agricultural output such as [orange, water melon, banana, apple, pineapple] have acquired extensive cultural and symbolic meaning. In common language usage ‘‘fruit normally means the fleshy seed associated structures of a plant that are sweet or sour and edible in the raw state, such as apples, oranges, banana, water melons, lemons, grapples.

Pathogens on fresh fruits are Salmonella, Shigella. Lasteria Monocytogenes, *Ecoli*0157: HI, gastrointestinal viruses, *Entamoebahistolystica* and Ascaris spp. usually this pathogens, are incorporated by polluted irrigated water. Fruits are generally too acidic for growth of the more common food borne pathogens such as salmonella and Shigella[in citrus juices]. Listeria monocytogens can survive well on both conditions.

The fruit is limited especially by its climatic requirement, with race differences. It is also highly susceptible to drought injury. But excess soil moisture is equally fatal, encouraging the dreadful *phytopthora*, and fungus growth. Despite the high water activity of most fruit the low pHleads to their spoilage being dominated by fungi, both yeast and moth.

The fruits have being estimated that one fourth of all product harvested is not consumed before spoilage of fresh fruit usually occurs during storage and transportation and while waiting to be processed unlike many other foods fruits after picking and before processing, microbiological spoilage of [orange, pineapple, water melon, apple, banana product [Samson et.al., 1988].

The composition of the fruit influences type of spoilage. Thus bacteria soft root is widespread for the most part among the fruit which is limited to those that are not highly acidic. Because most fruits are somehow acidic and are fairly dry at the surface and are deficient in B vitamins, moulds are the most common causes of spoilage, the compositions too, must determine the particular kind of moulds most likely to grow on orange, pineapple, banana, water melon, apple which support a large variety of fungal growth. Frankhauser, D.B (2005).

OBJECTIVES

- To identify how micro-organism destroy fruits so as to guide producer and farmers on how to manage fruits.
- To isolate some micro-organisms responsible for the spoilage of the fruit such as [orange, banana, pineapple, apple, water melon, paw-paw].
- To compare the extent of spoilage in the area of collection with that of market.

METHODOLOGY

Five (5) piece each of pineapple, orange, banana and some apples were purchased at four different markets, namely: Naibawa Yanlemo Market, Yankaba Market, Jakara Market and Sharada Market within Kano metropolis.The orange, banana, pineapple and apple samples collected were all fresh undamaged healthy and ripe. The samples were dispensed into well clean edpolythenebags and were brought to the laboratory. The samples were left free of dust, insect and were under room temperature for one week to undergo natural process of spoilage before being used for the study.

The media (Dextrose agar) was prepared strictly according to manufacturer's instruction. They were sterilized by auto autoclavingat 121^0c for 15 minutes. After sterilization the agar was allowed to cool down to a temperature of 40^0cand was poured into appropriate petri-dishes. Culture media used were nutrient agar for bacteria and sabourauddextrose agar [SDA] for fungi.

SAMPLE ANALYSIS

Samples were blended using a sterile blender. A homogenate of each sample were made by blending ten grams in 25ml of sterile water and then the samples were blended once more to remove all big particles. Serial dilutions of up to 10^{-1}-10^{-5} were made in sterile test tubes by several transfers of 1ml of previously diluted samples from one dilution tube to 9ml of sterile water in another tube.

INOCULATION AND INCUBATION

After preparation of serial dilution up to 10^{-1}-10^{-5}, then 1ml of serially diluted fruit sample was pipetted out to each serially marked petri-dish. Nutrient sabourauddextrose agars were poured into appropriately marked plates. The nutrient agar plate was then incubated at a temperature of 37^0c for 24hours, while the SDA was left at room temperature for 5 days. (Check your methodology under the sub heading here and compare with what you have on paragraph 2 under methodology; you need to state clearly the method you used for the study i.e pour plate, streak plate, swab etc).

FUNGI ISOLATION DISTINCT COLONIES FROM THE SDA

SDA agars were sub-cultured into freshly prepared agar using aseptic techniques to prevent contamination. The plates were incubated at room temperature for 72 hours for the fungi and at 37ºC for 24 hours for bacteria. The developed colonies were counted and colonies forming units were calculated and recorded. The colonies were purified and then later stored in nutrient agar slant in refrigerator [4^0c] for characterization. Cultural characteristics (colour, margin and shapes of the bacteria and fungi on the media were observed and recorded).

STAINING TECHNIQUES FOR BACTERIAL ISOLATION [GRAM STAIN]

The Gram Stain was carried out on 24hours cultures. A smear of each of the bacteria and fungi isolates was made on clean grease free slide and heated using flame. Crystal violet stain [0.3% w/v] was added and allowed to stand for one minute. The stain was washed off with distilled water iodine [0.4% w/v], a mordant was added and allowed to stand for one minute before being rinsed off with distilled water. Ethanol [95% w/v], a decolouriser was then added and allowed to stand for 30 seconds before being rinsed off with distilled water and then counter stained with the secondary stain, Safranin [0.4% w/v] and allowed to stand for one minute. This was then

washed off with distilled water and allowed to dry. The stained smear was then observed under the microscope using oil immersion lens magnification [x100].

BIOCHEMICAL TEST FOR IDENTIFICATION OF BACTERIA ISOLATES [INDOLE TEST]

The test organism was inoculated into a broth that contained tryplophan and incubated at 37^0c for 48 hours. Then 2ml of the broth suspension was transferred to another test tube under aseptic condition about 0.5ml of Kovac's reagent was added to the broth. The mixture was shaken properly to ensure a thorough mixing and then observed for colour reaction. A positive result was indicated by a pink-coloured ring round the interface between the broth suspension and alcohol regant which roseto the surface.

RESULT AND DISCUSSIONS

The cultural morphological and biochemical characteristics of the isolated bacteria found in all fruits samples purchased from markets. The probable (how sure is the result if the organisms isolated are probable species) species which were identified include; micrococcus sp Bacillus sp, Enterobactersp, Acinetobactersp, Citrobactersp etc.

The result of the total heterotrophic count of bacteria and fungi present in spoilt fruit purchased fresh from markets are shown table 1. The total heterotrophic bacteria count range from 4.2-6.1 x 810^4cfu/g, while the total heterotrophic fungal count ranged from 3.3 to 3.9 x 10^4cfu/g in group A fruit.

While in group B, fruit sample had a total heterotrophic bacteria count range from 5.6-8 x 10^4cfu/g, while the total heterotrophic fungal count from 2.1 – 4.810^4cfu/g. Also in the last group of fruitthe total heterotrophic bacteria count range of 4.3 – 6.8 x 10^4cfu, the bacterial count range from 4.9 – 5.5 x 10^4cfu/g (conflicting: which count is for bacteria and which for fungi since there are three ranges of counts?) and 1.9 – 3.2 x 10^4cfu/g respectively.

Table 1;

The mean heterotrophic bacterial and fungal counts of fruit purchased from the markets.

The highest mean bacterial count from all the sampled market was at 6.7 x 10^4cfu/gfor the second group of fruit respectively.

The mean fungal count of 3.6 x 104cfu/g was the highest and found in fruit sample from the 1st group of fruit. The lowest mean count of 2.6 x 104cfu/g was obtained from the last group of fruit collected.

Table 1; Heterotrophic bacterial and fungal count of fruits purchased from market.

Sampling group	Total heterotrophic bacterial count after 48hrs] 104cfu/g	Total heterotrophic fungal count after 5 days 104cfu/g
GROUP A		
A1	4.8	3.9
A2	5.8	3.3
A3	6.8	3.8
GROUP B		
A1	8.6	2.1
A2	6.1	2.8
A3	5.6	4.8

GROUP C		
A1	6.8	3.2
A2	6.1	2.4
A3	4.3	4.4
GROUP D		
A1	5.5	2.8
A2	5.4	1.9
A3	4.9	3.2

Key:
Where A1 – A3 represents the number of time sampling was done.
N/A: Not Applicable
+: Positive
-: Negative
Table 2: Morphological and cultural characteristics of fungal isolates. The fungal species isolated were; Saccharomyces spp and Geotrichum spp.

Table 3; Morphological and cultural characteristics of fungal isolates.

Isolates	**Macroscopy**
Mucor SP (spp)	Cover agar surface. Sparsely septate broad They are white and hyphae sporangiophores, Fluffy that later turned sporangia and spore. Grey, reverse side is white were visualized.
SaccharoyceteSp (spp) (Saccharomyces)	Colonie of Saccharomyces Unicellular, globose and grow rapidly. They are clipiod elongate in shape. Feet, smooth, moistglisteringAnll and cream to tanish Cream in colour
GeotrichumSp (spp)	Colonies of Geotrichum Sp. Unicellular in chains, Produced rapidly growing, lryalline and resultWhite, dry and powdery to from fragmentation Cottony colonies resembling of undifferentiated Ground grass.Hypae by fission through double septa. They are rounded at the end.

The rapid proliferation of both bacteria and fungi within the days of storage show that the adequate nutrients were available for microbial growth see (table 1) However, a phenomenon possibly due to exhaustion of nutrients for sustainability. Frazier and Weshoff (1998) reported that the availability of nutrients is crucial to increase or decrease of microbial numbers in fruits during spoilage.
Microorganisms are ubiquitous and they have been found to colonize fruits due to their high nutritional content that can support their growth and cause spoilage. The result from this research identified seven bacteria isolate, namely Bacillus sp, enterobacter sp, citrobecter sp, acinetoacter sp, micrococcus sp, acinebacter sp, klebsiella sp and bacillus spalso three fungi isolates, saccharomyces sp, mucor sp and geotrichum sp, were found in the sample collected from different markets.

The bacteria species, micrococcus sp and Klebsiella isolated from spoilt fruit in this study were also identified in a similar investigation carried out by Ikenebomeh and Chikneedu (1997). These organism have been reported as the causative agents for bacterial soft rots. Like fungi, they can hydrolysespectin giving rise to(reframe) a soft mushy appearance or consistency (Liao et al, 1993). Their presence(is) suggestive of contamination from soil, harvesting equipment, handling and storage facilities and non-food – contact surfaces throughout the distribution chain. Gram negative rod-shaped bacteria such as *Acinobacter* sp may also grow at Chili temperatures and have been shown to contribute to the spoilage of post – harvest fruit. The coliform enteric bacteria e.g citrobacter sp and *Enterobacter* sp, generally slower in growth atchill temperature become more significant as the temperature rises above 5^0c. Their spoilage action is characterized by the production of gas, acid, slime, bitter flavors and faecal odours.

Bacillus sp has been reported to(be)the most antagonist (antagonistic)microorganism on post-harvest of fruits. This is in line with the findings of Korsten et al, (1993). Colonization of fruits and vegetable by the invading microorganism is a critical phases in the microbial spoilage of produce. The colonization process involves the ability of the microorganism to establish themselves within the produce. This is initiated when the microorganisms following adhesion and release of enzymes degrade certain specific cell wall polymers such as protopectin, the cementing substance of the produce. The magnitude of the symptoms of the induced disease is a reflection of the extent of colonization (Chukwu et al., 2008) whereas both fruits and vegetables are highly susceptible to microbial spoilage. There is a variation in the susceptibility which is due largely to the differential chemical composition such as PH and moisture contents. Thus, the lower pH and moisture contents of the fruits make them more prone to fungal spoilage. Efiuvwevewer (2000), also reported that high moisture and relative humidity led to greater fungal growth in agricultural produce and thus low storability of fruits and vegetables.

CONCLUSIONS.

The prevalence of fungi as the spoilage organism of fruits is due to a wide range of factors which are encountered at each stage of handling from pre-harvest to consumption and is related to the physiological and physical condition of the produce as well as the extrinsic parameters to which they are subjected. Geotrichum candidum isolated in this study are among the fungi responsible for post harvest rot of the samples. This finding is in agreement with the reports of previous investigations carried out by Akande (1975) and Onesira and Fatula (1976) in South Western Nigeria. Chukwu et al (2008) also identified similar fungi from tomato and snake gourd in Rivers State Nigeria. The fungal isolates in this study geotrichumsp, Saccharomyces sp and Mucorsp probably grew due to the amount of oil found in fruits as suggested from the reports of previous investigations. These organisms present a formidable challenge to commercial fresh fruit product operations from the farm to retail and wholesale outlet (Liao et al, 1993, 1997).

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