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Micro-Organisms Associated with Spoilage of Sweet Potatoes Sold at Major Markets in Owerri, Nigeria.

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ABSTRACT: Sweet Potatoes are tuber crops consumed in different parts of the world. The implication of microbial contamination and growth on sweet potato produce causes post- harvest spoilage which reduces market appeal, decreases shelf and wastage of the product which in turn has serious health consequences. This study was carried out to isolate and identify the micro-organisms responsible for the post-harvest spoilage of sweet potato. About Fourty -five(45)samples of decaying sweet potato were obtained randomly from the major markets (Ekeonuma, Relief and World bank markets) in Owerri. The samples were homogenized and serially diluted to "thin out" the microbial population. Nutrient agar and Potato dextrose agar were used for the isolation of the bacteria and fungi respectively. The bacterial population was estimated after 24hrs of incubation while fungi were obtained after 7 days of incubation. The organisms were identified using standard morphological and biochemical tests .The total aerobic bacterial count of the samples ranges from 2.3 x10^6 cfu/g to 4.3 x 10^6 cfu/g while the fungi load ranges from 0.8 x 10^5sfu/g to 1.4 x 10^5sfu/g. The genera of the bacteria isolated and their percentages of occurrence include Bacillus substilis (18.8%) Bacillus licheniformis (9.4%),Micrococcus luteus(3.1%), Micrococcus varians(6.3%), Klebsiella pneumoniae(21.9%),Pseudomonas fluorescens (9.4%) and Staphylococcus auerus(31.3%) while the genera of fungi isolated and their percentages of occurrences include; Aspergillus niger (70%), Mucor spp(23%) and Fusarium spp(7%).The micro-organisms gained entry into the sweet potato majorly through openings on the tubers caused by several physical injuries and are able to proliferate because of the temperature and the humidity of storage condition of the tuber. The micro-organisms then breakdown the starch present in the tuber and therefore leads to spoilage of Sweet potato.Due to the considerable economic importance of sweet potato tubers,their safe storage after harvest is of great concern.

Keywords: Sweet potato, Post-Harvest Spoilage, Micoorganisms, Physical injuries, Storage

I. INTRODUCTION

Sweet potato (Ipomea batatas) is one of the most important crops worldwide including wheat, rice, maize, potato and cassava (Truong et al., 2011). Sweet potatoes possess many positive health benefits including sources of anthocyanins, phenolic compounds and other bioactive compounds (Giusti and Wrolstad, 2003).

It is an economic source of food energy and also possesses antioxidant activities (Oludoye et al., 2013). The crop tubers have been consumed in various forms in developing countries of Africa, including eating in cooked form, incorporation with yam during pounded yam preparation, processing into flours along with yams for edible dough (such as amala in Nigeria) etc.

Food spoilage is a metabolic process which may be brought about by microbial action and causes foods to be undesirable or unacceptable for human consumption due to deterioration in quality characteristics. Sweet potatoes have been described as having thin, delicate skin that is easily damaged by cuts and abrasion during harvesting, transportation or distribution. It has been reported by some workers that the micro – organisms that are responsible for the spoilage of sweet potato produce extra – cellular enzymes such as amylases, cellulose,

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polygalactunases, cylanases, and pectin – methyl esterases and these enzymes degrade the cell wall components of produce that are susceptible leading in some cases of emission of offensive odour and water (Salami and Popoola, 2007).

Enormous post harvest losses have been attributed mainly to fungal deteriorations (Okigbo, 2003). Fungal pathogens cause spoilage and post – harvest spoilage of sweet potato by producing various types of mycotoxins. Several fungi have been implicated in the spoilage of sweet potatoes. In (2002), Onuegbu reported Penicillium sp, Ceratocystis timbriata (black rot), Aspergillus niger, Diaporthe batatalis, and Aspergillus flavus as fungi responsible for the post – harvest decay of sweet potato. In some instances, bacteria (Pseudomouas and Erwinia) may play associative roles in rots of vegetables. Only 36% of postharvest rots of vegetables are attributed to bacteria (Agriculture information bank, 2013). Staphylococcus acuri and Rabniell sp were associated with spoilage of vegetable sweet potato based on the DNA sequencing studies in south-western Nigeria (oladoye et al., 2013).

The objectives of this study were to isolate identity and characterise the fungi and bacteria that are possibly responsible for the spoilage of sweet potato. This research work is carried out in response to an increase in post – harvest spoilage of sweet potato in Owerri, Imo State.

II. MATERIALS AND METHODS

2.1 Sample Collection

About fifteen (15) samples of decaying sweet potato were obtained at different locations of the three main markets (Eke –onuwa, Relief and World Bank markets) in owerri. They were collected in a sterile polythene bags and were brought into the laboratory in the Department of medical laboratory science, Imo state university owerri for microbial analysis.

2.2 Preparation and Sterilization of Media

The preparation and sterilization of media, water and glasswares for the experiment were properly done before the collection of the sample. Before sterilization, glassware were thoroughly washed and rinsed with 70% ethanol. The pipettes were wrapped with foil and sterilized in a hot air oven. Nutrient agar, MacConkey agar and potato dextrose agar (PDA) were used. The media were prepared according to the manufacturer's instructions. Chloramphencol (50mg/l) and cycloheximide (500mg/l) were added aseptically to the molten PDA after sterilization .When the medium has cooled to inhibit the growth of bacteria and prevent saprophytic fungal contamination.

2.3 Preparation of Samples

Cotton wool moistened with 70% ethanol was used to sterilize the workbench, hands and knife. The sterile knife was then used to cut the sample open and the decaying inner part was cut into pieces. A sterile pestle and mortar were used for homogenization. A weighing balance was then used to weigh one gram of the homogenised solid.

2.4 Isolation of Micro-organisms from Samples

Using the method of Harrigan and McCance (2005), one gram of each sample was poured into 10mls of sterile distilled water and the tube was properly mixed. After mixing, a serial dilution was then carried out by transferring 1 ml from the first tube into the second test tube that contains 9mls of distilled water. The second tube was mixed gently and 1ml was taken from the second tube test – tube and so on till the fifth test–tube.

Isolation of organisms was done using the pour plate method (Harrigan and McCane, 2005). One mililiter (1ml) each from the third test–tube was pipette using a new sterile pipette into three empty sterile petri–dishes, one for nutrient agar, one for MacConkey agar and the other for potato dextrose agar. Also, the same process was repeated with the sample from the fifth test–tube. After the samples have been poured into the petri – dishes, the media (about 15 mls) were poured into their respective plates. After the plates had set nutrient agar and MacConkey agar were incubated at 37^{o}c for 24 hrs while the potato dextrose agar plates were incubated at 25^{0}c for 3 – 5 days.

2.5 Purification and Identification of Isolate

Predominant micro – organisms of morphologically, different, colonial types were purified with nutrient agar and MacConkey agar. The purified isolates were sub cultured in agar slopes and then cultures were stored in the refrigerator at 4^{0}c, until used for further tests. The identification of purified isolates were carried out according to (Cheesbrough, 2000)

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2.6 Lactophenol Cotton Blue Stain

A drop of lactophenol cotton blue stain was placed on clean grease – free slide. A small portion of the filamentous part of the fungal isolates was emulsified in the stain. Then, the slide was covered with a covership avoiding bubbles. The slide was then viewed under the microscope. This technique shows various microscopic characteristics of the fungal isolates (Onuorah et al., 2015).

2.7 Spore Staining

A heat fixed smear from a 24 hour old culture was prepared on grease – free slide and was covered with a square of blotting paper. The blotting paper was saturated with malachite green stain solution and was steamed for 5 minutes, keeping the paper moist and adding more dye as required. The slide was then washed in tap water. Safranin was used to stain the smear for 30 seconds and was washed with tap water and blotted dry. The slide was then examined under oil immersion lens (x100) for the presence of endospores. Endospores are bright green and vegetative cells are brownish red to pink (Onuorah et al., 2015).

2.8 Citrate Utilization Test

A dense bacterial suspension of the test – organism in 0.25ml sterile physiological saline is prepared in a small tube. A citrate tablet is added and the tube was stoppered. It was incubated overnight at 37^0c.The technique assists in the identification of enterobacteria. The presence of red colour shows positive citrate test while yellow – orange colour shows negative citrate test (Cheesbrough, 2000).

Other tests carried out include; methyl red,voges – proskauer, indole, starch hydrolysis, casein hydrolysis, sugar fermentation, Gram's staining, catalase test, oxidase test, coagulase test etc.

III. RESULTS

The microbial load (mean value) of fourty five different samples of sweet potatoes obtained within Owerri is presented in Table 1. The total aerobic bacteria count was measured in colony forming unit per gram (cfu/g) while the total fungal count was measured in spore forming unit per gram (sfu/g). In general, the bacterial load was more than the fungal load. The morphological characteristics of the bacterial and fungal isolates are shown in table 3 and table 4 respectively. The biochemical characteristics of the bacterial isolates are shown in table 7 while the description and the probable identity of the fungal isolates are shown in table 5. The probable identity of the microbial isolates is listed after examining several characteristics. The percentage occurrences of the fungal isolates are shown in table 4. Among the genera of the bacteria identified, the genus Staphylococcus aureus was most predominant, followed by Klebsiella pneumoniae and Bacillus subtilis.

Table 1 Mean Microbial Load of Samples

Market	No. Of samples	Total bacterial count (mean value) cfu/g	Total fungal count (mean value) cfu/g
Relief	15	2.3×10^6	1.1×10^5
Douglas	15	4.3×10^6	1.4×10^5
World bank	15	3.2×10^6	0.8×10^5

Table 2 shows the Percentage & Mean Counts of different Bacterial Isolates expressed in Cfu/g

Bacterial Isolates	Relief Market	Douglas Market	World Bank Market	Mean count	Percentage (%)
Staphylococcus aureus	0.8×10^6	1.3×10^6	1.0×10^6	1.0×10^6	31.3
Klebsiella pneumoniae	0.5×10^6	0.8×10^6	0.7×10^6	$\mathbf{0.7} \times 10^6$	21.9
Bacillus subtilis	0.4×10^6	0.7×10^6	0.6×10^6	$\mathbf{0.6} \times 10^6$	18.8
Bacillus licheniformis	0.1×10^6	0.6×10^6	0.3×10^6	$\mathbf{0.3} \times 10^6$	9.4
Pseudomonas fluorescens	0.2×10^6	0.4×10^6	0.3×10^6	$\mathbf{0.3} \times 10^6$	9.4
Micrococcus varians	0.2×10^6	0.3×10^6	0.2×10^6	$\mathbf{0.2} \times 10^6$	6.3
Micrococcus luteus	0.1×10^6	0.2×10^6	0.1×10^6	$\mathbf{0.1} \times 10^6$	3.1

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Table 3 Morphological Characteristics of the Bacterial Isolates

S/N	Isolate code	Colour	Opacity	Elevation	Surface	Edge	Shape
1	BA1	White Yellow	Opaque	Raised	Smooth glistening	Entire	Circular
2	BA2	Off – white	Opaque	Flat	Dull/rough	Lobate	Irregular
3	BA3	Off-white	Opaque	Flat	Dull/rough	Lobate	Irregular
4	BA4	Grey white	Opaque	Raised	Smooth/glistening	Entire	Circular
5	BA5	Reddish grey	Opaque	Flat	Smooth/glistening	Lobate	Circular
6	BA6	Yellow	Opaque	Flat	Dull/rough	Lobate	Irregular
7	BA7	Yellow	Opaque	Raised	Smooth/glistening	Undulate	Irregular

Table 4 Morphological Characteristics of Fungal Isolates

S/N	Isolate code	Colour of spores	Reverse side of the agar	Aerial hyphae	Abundance	Growth	Pigmentation
1	FU1	Black	Light green	Powdery , spores embedded	Abundant	Fast	No
2	FU2	White	Green	Fluffy, raised a little	Abundant	Fast	No
3	FU3	White	Orange	Fluffy, raised	Abundant	Fast	No

Table 5 Percentage of Occurrence of Fungal Isolates from Forty Five (45) Samples

Fungal isolate	Frequency	Percentage (%)
Aspergillus niger	21	70
Mucor spp	7	23
Eusarium sp	2	7
Total	30	100

Table 6 Description of the morphological characteristics of fungal isolates

S/N	Fungi	Description
1	Aspergillus niger	They are typically powdery black. Conidiophores arising from long, broad thick-walled, sometimes branched foot cells. It has tall conidiophores. Conidia are large with radiating heads, mostly globose and irregularly roughed.
2	Mucor sp	Colonies are whitish to olivaceous –buff, odour aromatic; in the dark differentiated into tall and short sporangiophores. Sporangia blackish with ellipsoidal, pyriform or subglobose. Chlamydospores absent.
3	Fusarium sp	Colonies are fast growing, aerial mycelium sparse to abundant and floccose, becoming felted, white or peach, but with a violet tinge. Characteristic aromatic odour suggesting lilae

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TABLE 7 The biochemical characteristics bacterial isolates

Isolate Code	Gram reaction	Cell morphology	Catalase	Oxidase	Methyl red	Voges proskauer	Indole	Citrate utilization	Growth in 6.5% NaCl	Starch hydrolysis	Casein hydrolysis	Acidic medium (3.9)	Basic medium(9.2)	High temp (70°C)	Low temp (20°C)	Fructose	Raffinose	Mannitol	Glucose	Trehalose	Maltose	Lactose	Sucrose	Galactose	Xylose	Probable identity
BA1	+	C	+	-	+	+	-	+	+	-	+	-	+	-	+	+	+	+	+	(+)	+	(+)	D	-	(+)	*Staphylococcus aureus*
BA2	+	R	+	+	-	-	-	+	+	+	+	+	-	-	+	-	+	+	+G	(+)	-	+	+	+	-	*Bacillus subtilis*
BA3	+	R	+	+	-	-	-	-	+	+	+	(+)	(+)	-	-	-	-	-	+G	+	+	(+)	(+)	+	+	*Bacillus licheniformis*
BA4	+	R	-	-	-	V(+)	V(+)	+	-	-	-	-	-	-	-	-	-		+	-	+	V(+)	+	-	-	*Klebsiella pneumoniae*
BA5	-	R	+	+	+	+	-	+	(+)	-	+	-	+	-	+	-	+	+	+		+	-	-	D	(+)	*Pseudomonas fluorescens*
BA6	+	C	+	+	+	+	-	-	+	-	-	-	+	-	+	+	-	-	(+)	-	+	+	-	+	+	*Micrococcus luteus*
BA7	+	C	+	+	+	+	-	(+)	(+)	-	-	(+)	+	-	+	+	(+)	-	+	+	(+)	(+)	(+)	-	(+)	*Micrococcus varians*

KEYS: C = Coccus, R= Rod, + = positive , (+) = weak positive, - = Negative, D = delayed reaction, +G = positive with gas V(+)=variable & weak reaction

IV. DISCUSSION

The results obtained from the study have shown that a range of microbial species were associated with the decayed potato tubers. The genera of the bacteria isolated and their percentage of occurrence include: Staphylococcus aureus (31.3%), Klebsiella pneumoniae (21.9%), Bacillus subtilis (18.8%), Bacillus licheniformis (9.4%) Pseudomonas fluorescens (9.4%), Micrococcus varians (6.3%) Micrococcus luteus (3.1%) while the genera of the fungi isolated and their percentage of occurrence include: Aspergillus niger (70%), Mucor sp (23%) and Fusarium sp (7%).

The total aerobic bacterial count of the samples ranges from 2.3 x 10^6 cfu/g while the fungal load ranges from 0.8 x 10^5 sfu/g to 1.4 x 10^5 sfu/g. Oladoye et al., (2013) identified Staphylococcus aureus, Bacillus spp and Pseudomonas as some of the bacteria that cause spoilage of sweet potato and these bacteria have the abilities to produce enzymes that are capable of degrading sweet potato tissues. Aspergillus, Fusarium and Geotrichum were isolated and found to be responsible for the spoilage of sweet potato (Khatoon et al., 2016). In this study, Aspergillus niger was found to have the highest percentage of fungal occurrence and this is similar to the report of Torte et al., (2010) that Aspergillus flavus and Aspergillus niger to be abundant fungal species during post harvest storage of sweet potato.

However, some of the harvest faults that lead to the wounding of the tubers must have facilitated in the microbial colonization of the harvested tubers. Since the tubers were in contact with soil and the soil particles carried with them loads of biodeteriogens, therefore, soil must have been the source of the decayed micro – organisms. Soil is recognized as reservoir of macro organisms both pathogenic and non pathogenic. Aspergillus niger and Aspergillus oryzae were among the fungi isolation which are known to produce amylase that must have contributed to the hydrolysis of the carbohydrate component of the experimental sweet potato. These fungal isolates also produce proteases, which must have contributed to the hydrogen of the protein component of the potatoes and its eventual decay (Smith 1969). The metabolic products that are produced during the decay process impart decayed potato and since many microbial metabolites are toxic, the decayed tuber might become a health hazard.

The toxins produced by the fungal isolates are dangerous in small, quantities and presents extreme toxicity because of their resistance to heat. Fungal toxins contaminate food products and cause acute or chronic intoxications. This leads to a

reduction in the expectancy, worsen disease conditions in humans leading to 40% loss of economic productivity (Okigbo, 2014; Shuklaa et al., 2012)

Since sweet potatoes have been described as having thin, delicate skin that is easily damaged by cuts and abrasion during harvesting, transportation or distribution, concerted effort should be directed towards minimizing or reducing this so as to avoid physical damage to the tuber and thereby reducing or preventing microbial attack (Rupsa et al., 2017). Low temperatures and high relative humidity in the post – harvest environment have been reported to affect the activity of decay causing organisms (Sholberg et al., 2001). Besides good handling and transportation practices, sweet potatoe tubers should be stored in an environment which discourages the growth of spoilage organisms particularly molds.

Sweet potatoes remain a significant crop for many countries and it is hereby suggested that fresh efforts should be made towards the reduction of post – harvest spoilage or wastage.

V. CONCLUSIONS

This work showed that both bacteria and fungi are responsible for the spoilage of sweet potato during storage. Several control techniques such as washing of the harvested tuber, cleaning of transit containers, proper handling of the harvested tubers to avoid injuries, lower temperature and low humidity on storage and others should be put in place. Due to the considerable economic importance of sweet potato tubers, their safe storage after harvest is of great concern.

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Interpretation of Research Findings

After collecting and analysing the data, the researcher has to accomplish the task of drawing inferences. This has to be done very carefully, otherwise misleading conclusions may be drawn and the whole purpose of doing research may act vitiated. It is only through interpretation that the researcher can expose relations and processes that underlie his findings. In case the researcher applied hypothesis testing, the explanations of his findings will be done on that basis.

Meaning of interpretation

Interpretation refers to the task of drawing inferences from the collected facts after an analytical and or experimental study. In fact, it is a search for broader meaning of research findings .The task of interpretation has two major aspects viz, (i) the effort to establish continuity in research through linking the results of a given study with those of another, and (ii) the establishment of some explanatory concepts. Interpretation is concerned with relationships within the collected data and the extension of study beyond the collected data as well. Thus, interpretation is the device through which the factors that seem to explain what has been observed by researcher in the course of the study can be better understood and it also provides a theoretical conception which can serve as a guide for further researches.

Interpretation is essential for the simple reason that the usefulness and utility of research findings lie in proper interpretation. It is being considered a basic component of research process because of the following reasons:

(i) It is through interpretation that the researcher can well understand the abstract principle that works beneath his findings. Through this he can link up his findings with those of other studies, having the same abstract principle, and thereby can predict about the concrete world of events. Fresh inquiries can test these predictions, maintaining continuity in research.

(ii) Interpretation leads to the establishment of explanatory concepts that can serve as a guide for future research studies; it opens new avenues of intellectual adventure and stimulates the quest for more knowledge.

(iii) Researcher can better appreciate only through interpretation why his findings are what they are and can make others to understand the real significance of his research findings.

(iv) The interpretation of the findings of explanatory research study often results into hypotheses for experimental research and as such interpretation is involved in the transition from exploratory to experimental research.

Techniques of Interpretation

The task of interpretation is not an easy job; rather it requires a great skill and dexterity on the part of researcher. Interpretation is an art that one learns through practice and experience. The researcher may, at times seek the guidance from experts for accomplishing the task of interpretation.

The techniques of interpretation often involve the following steps:

(i) Researcher must give reasonable explanations of the relations which he has found and he must interpret the lines of relationship in terms of the underlying processes and must try to find out the thread of uniformity that lies under the surface layer of his diversified research findings.

(ii) Extraneous information, if collected during the study, must be considered while interpreting the final results of research study, for it may prove to be a key factor in understanding the problem under consideration.

(iii) It is advisable before embarking upon final interpretation, to consult someone having insight into the study and who is frank and honest and will not hesitate to point out omissions and errors in logical argumentation.

(iv) Researcher must accomplish the task of interpretation only after considering all relevant factors affecting the problem to avoid false generalization.

Precautions in Interpretation

One should always remember that even if the data are properly collected and analysed, wrong interpretation would lead to inaccurate conclusions. It is therefore absolutely essential that the task of interpretation be accomplished with patience in an impartial manner and in correct, perspective. Hence

the following points are essential for correct interpretation:

(i) The researcher must invariably satisfy himself that (a) the data are appropriate, trustworthy and adequate for inferences; (b) the data reflect good homogeneity; and that (c) proper analysis has been done through statistical methods.
(ii) He must always keep in view that the task of interpretation is very much intertwined with analysis and cannot be distinctly separated. As such he must take the task of interpretation as a special aspect of analysis, and accordingly must take all those precautions that are usually observes while going through the process of analysis via; precautions concerning the reliability of data, computational checks, validation and comparison of results.
(iii) He must never lose sight of the fact that his task is not only to make sensitive observations of relevant occurrences but also to identify and disengage the factors that are initially hidden to the eye.