Isolation And Identification Of Microorganisms Associated With Spoilage Of Cabbage (*Brassica Oleracea)* In Sabon-Gari Market Kano Nigeria

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ABSTRACT

Microorganisms associated with spoilage of Cabbage (*Brassica oleracea*) were studied using standard microbiological methods (Gram staining, bacteriological analysis and Biochemical tests) for bacteria and (macroscopic and microscopic examination) for fungi. The analysis was done on fifty (50) samples each of cabbage with soft rot symptoms purchased from different vendors within Sabon – gari Market, Kano. The result obtained from the data shows that the bacteria found in spoiled cabbage samples include *Bacillus* (19%), *Klebsiella* (10%), *and Escherichia coli* (15%), *Staphylococcus* (26%), *Salmonella* (20%) and *Pseudomonas* (10%). This shows *Staphylococcus* was the highest occurring organism (26%) while *Pseudomonas spp* is the least occurring (10%). It also showed that of the three fungi species were isolated from spoiled cabbage, *Rhizopus stolonifer* (31%) and *Aspergillus spp* (34%) each and Penicillium (32%).

Keywords: Microorganisms, spoilage, Cabbage, Pathogenicity.

INTRODUCTION

Most of our foods are excellent source for rapid microbial growth. Food materials contain organic substances in plenty and sufficient amount of water, and they may be either neutral or slightly acidic in nature (Singh *et al*., 2013). They are subjected to natural contamination by many different kinds of microorganisms, including pathogens. Metabolic activities of microbes alter the condition of food, resulting in its spoilage (Angela *et al*., 2010). The airborne microbes fall on fruits and vegetables and enter through the ruptured skin. The microorganisms present in the soil reach the processing plant through the crops (Neeraj and Sharma, 2007). Several insects are also responsible for the transference of microbes to the food. In general, the keeping quality of food depends on the success of preventing the entry of micro-organisms and restricting their growth (Sing *et al*., 2013).

Foods and microorganism have long and interesting association which developed long before the beginning of recorded history (Sing *et al*., 2013). Foods are not only nutritious to consumers, but are also excellent source of nutrients for microbial growth. Depending upon the microorganism present, foods may spoil or be preserved by fermentation (Neeraj and

Sharma, 2007). Fruits and vegetables are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibres for human and are thus vital for health and well being (Angela *et al*., 2010). Vegetable are widely exposed to microbial contamination through contact with soil, dust, water, and by handling at harvest or during post harvest processing. They therefore harbor a diverse range of microorganism including plant and human pathogens (Dunn *et al*., 1995; Carmo *et al*., 2004). Differences in microbial profile of various Vegetables result largely from unrelated factors such as resident micro flora in the soil, application of non-resident micro flora via manure, sewage or irrigation water, transportation and handling by individual retailer (Ray and Bhunia, 2007). In developing countries such as Nigeria, continuous use of untreated waste and manure as fertilizer for the production of vegetable is a major contributing factor for contamination (Olayemi, 1997; Amoah *et al*., 2009). In this study, a vegetable; Cabbage (*Brassica oleracea*) were examined for the isolation and identification of the microorganisms associated with their spoilage and the rate at which the organisms causes the spoilage.

MATERIALS AND METHODS

Study area

The study area was Sabon – gari market in Fagge local government area of Kano state Nigeria. The research is carried out between months of August to December meaning that the recent weather is rainy season which extended to harmattan season.

Collection of samples

Fifty (50) samples each of cabbage were purchased from different vendors within Sabon gari market Kano. The spoiled samples were taken to the laboratory for use. The decaying portion was subjected for bacterial and fungal identification (Anitha *et al*., 2014).

Isolation of bacteria

Nutrient agar was prepared according to Manufacturer's instruction, and sterilized by autoclaving at 121°C for 15mins. Five grams (5g) of each spoiled sample was weighed and washed in 10 ml of sterile distilled water. Nutrient agar plate was inoculated with 1 ml of the rinse water using the Pour Plate Technique (for bacteria) The plates were allowed to solidify, inverted and incubated at 37ºC for 24hrs for bacterial and fungal colony formation. Each colony was isolated in a pure form by sub culturing in a fresh nutrient agar plate for further studies and identification. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Further microbial identification was based on the methods of Jolt *et al*., (1994).

Isolation of fungi

A segment of 3cm from the tissues from the margins of the decayed cabbage samples were cut with a sterile scalpel and placed on the potato dextrose agar in Petri dishes and incubated at 37°c for 3 days for fungal growth. Each colony was isolated in a pure form by sub culturing in a fresh potato dextrose agar plate for further studies and identification

Bacterial identification

The discrete colonies from these sub cultured plates were identified by Bacteriological Analysis

using selective media method as described by Presscott, (2002) and Sherman, (2005), Morphological Characterization on the basis of simple staining and gram staining (Holt *et al*., 1994; Sherman, 2005) and set of Biochemical Characterization i.e. indole test, Methyl-Red test, Vogues-Proskauer test and Citrate utilization test, catalase test, coagulase test and oxidase test by standard method given by Sherman, (2005) and Holt *et al*. (1994).

Fungal identification

The pure isolated fungi were identified using cultural and morphological (microscopic) features according to the most documented keys in fungal identification (Domsch *et al*., 1993).

RESULTS AND DISCUSSION

Bacterial Isolation and identification

Morphological Characterization

Morphological characterization of recovered isolates recovered from ten spoiled cabbages samples is presented in Table 1. Colonial morphology and gram staining of each isolate was recorded which is used to predict suspected organism. A total of 37 isolates were recovered from the ten spoiled cabbages samples

Table 1: Morphological characterization of the recovered bacterial isolates

ISO. CODE	COLONY MORPHOLOGY	G/STAINING	SUSPECTED ORG
C_1	Produce opaque cream yellow growth	Positive/cocci	*Staphylococcus spp*
C_2	Produce large irregular flat growth	Positive/Rod	*Bacillus spp*
C_3	Produce colorless colony	Negative/Rod	*Salmonella/Shigella*
C_4	Produce shiny mucoid/viscous colony	Negative/Rod	*Klebsiella spp*
C_5	White glistering and moist growth	Negative/Rod	*Escherichia coli*
C_6	White growth turning media light green	Negative/Rod	*Pseudomonas spp*

Biochemical Characterization

The result of biochemical characterization of the recovered isolates is represented in Table 2. Tests conducted include indole, Methyl red, Vogues Proskauer, citrate utilization, catalase, oxidase, and coagulase test, lactose and Mannitol fermentation.

Table 2: Biochemical characterization of the recovered bacterial isolates

	IN	MR	VP	CI	CA	OX	CO	MF	LF	SUSP. ORGANISM
C_1	-	+	+	-	+	-	+	+	-	*Staphylococcus spp*
C_2	-	-	-	-	+	+	-	-	-	*Bacillus cereus*
C_3	-	+	-	+	+	-	-	-	-	*Salmonella spp*
C_4	+	+	-	-	+	-	-	+	-	*Klebsiella spp*
C_5	+	+	-	-	+	-	-	+	-	*Escherichia coli*
C_6	-	-	-	+	+	+	-	-	-	*Pseudomonas aeruginosa*

IN=Indole, MR=Methyl Red, VP=Vogues Proskauer, CI=Citrate, CA=Catalase, OX=Oxidase
CO=Coagulase, LF=Lactose Fermentation, MF=Mannitol Fermentation

The number and percentage occurrence of the recovered bacterial isolates is presented in Table 3. *S. aureus* has the highest number of occurrences, ten times which accounted for 26% while *Pseudomonas spp* and *Klebsiella spp* has the least appearance i.e. 4 accounted for 10%. Both *Bacillus cereus* and S. *typi* appeared eight and six times respectively.

Table 3: Number and percentage occurrence of bacterial isolates in spoiled cabbage samples

BACTERIA	NO. OF OCCURANCE	% OCCURANCE
Staphylococcus spp	10	26%
Bacillus cereus	08	20%
Salmonella spp	06	15%
Klebsiella spp	07	19%
Escherichia coli	04	10%
Pseudomonas aeruginosa	04	10%
TOTAL	**39**	**100%**

Fungal isolation and identification

Macroscopic Examination

Macroscopic (morphological) examination of recovered fungal isolates from ten spoiled water melon samples is represented in Table 4. Colonial morphology of each isolate was recorded which is used to predict suspected organism. A total of 29 isolates were recovered from 10 spoiled cabbages sample.

Table 4: Macroscopic examination of fungal isolates recovered from ten spoiled cabbage samples.

ISOL. CODE	COLONY MORPHOLOGY	EXPECTED ORGANISM
C_1	White cottony growth on PDA plate	*Rhizopus stolonifer*
C_2	Blue-black mould on PDA plates	*Aspergillus spp*
C_3	Blue mold – rot on PDA plate	*Penicillium spp*

Microscopic Examination

Microscopic examination of recovered fungal isolates from ten spoiled cabbage samples is represented in Table 5. Microscopic appearance of each isolate was recorded which is used to predict suspected organism.

Table 5: Microscopic examination of fungal isolates recovered from ten spoiled cabbage samples.

ISOL. CODE	MICROSCOPIC APPEARANCE	EXPECTED ORGANISM
C_1	Presence of stolon and rhizoid with sporangia above rhizoid	*Rhizopus stolonifer*
C_2	Conidiophores terminate in a ball like structure	*Aspergillus spp*
C_3	Chain on finger-like projection from conidiophores	*Penicillium spp*

The number and percentage occurrence of the recovered fungal isolates is presented in Table 6. *Rhizopus stolonifer* and *Aspergillus spp* has the highest number of occurrences, ten (10) times which accounted for 34% while *Penicillium spp* has the least appearance (9) accounted for 32%.

Table 6 Number and percentage occurrence of bacterial isolates in spoiled cabbage samples

FUNGI	NO. OF OCCURANCE	% OCCURANCE
Rhizopus stolonifer	10	34%
Aspergillus spp	10	34%
Penicillium spp	09	32%
TOTAL	**29**	**100%**

Pathogenicity test

The Pathogenicity of the fungal isolates on fresh and healthy cabbage is presented in Table 7. All the tested isolates were found to be pathogenic on the cabbage. *Rhizopus* is found to be most pathogenic with diameter of the spoiled area of 5mm while *Penicillium spp* and *Aspergillus spp* recorded 4 mm each. No spoilage was formed in the control water melon fruit used.

Table 7: Pathogenicity of fungal isolates on healthy cabbage

Test isolates	Inoculated sample diameter after 4 days (mm)	Texture of the inoculated spoiled area	Spoiled diameter of control after 4 days (mm)	Texture of control after 4 days
Rhizopus stolonifer	**5**	**Rot**	**3***	**Turgid**
Aspergillus spp	**4**	**Soft**	**3***	**Turgid**
Penicillium spp	**4**	**Soft**	**3***	**Turgid**

3* = no spoilage

The current study aimed at reporting the microorganism responsible for spoilage of cabbage at different points of sale at Sabon – gari market in Kano metropolis. A total of sixty eight (68) microbial isolates were recovered out of which thirty nine (39) are bacteria while twenty nine (29) are fungi from the ten (10) spoiled cabbages samples. The thirty nine (39) recovered bacterial isolates were characterized on the basis of colony morphology and the staining characteristics (Table 1). It was observed that twenty two (22) isolates were gram negative, while fifteen (15) isolates were gram positive. Bacteriological characterization of gram negative isolate was conducted using MacConkey agar to test their ability to ferment lactose and produce acid. Out of twenty two gram negative isolates only ten were able to ferment lactose and produce acid while twelve do not. The thirty nine bacterial isolates were characterized on the basis of biochemical tests. The result obtained from the data shows that the bacteria found in spoiled cabbage samples include *Bacillus* (19%), *Klebsiella* (10%), *and Escherichia coli* (15%), *Staphylococcus* (26%), *Salmonella* (20%) and *Pseudomonas* (10%). This shows *Staphylococcus* was the highest occurring organism (26%) while *Pseudomonas spp* is the least occurring (10%). On the other hand, twenty nine (29) fungal isolates were recovered from ten spoiled cabbage samples, both macroscopic and microscopic examination of the isolates shows that three different species of fungi were found. These include *Aspergillus, Rhizopus and Penicillium* and their prevalence was 34%, 34% and 32% respectively. *Rhizopus* and Aspergillus were the highest occurring organisms with 34% each while *Penicillium* is the least occurring with percentage prevalence of 32%.

This result was in conformity with several studies conducted by many researchers in an attempt to determine or identify the microbial population responsible for vegetable spoilage. For example, a study conducted by Manani *et al.,* (2006) among the vegetable to identify bacteria causing spoilage, eight samples was collected and twenty-one isolates were recovered from them. The bacteria found to spoil the vegetables were identified as *Klebsiella, Bacillus, E. coli, Staphylococcus, Pseudomonas,* and there prevalence in vegetable sample was found to be 33.33%, 23.80%, 14.28%, 14.28% and 14.28% respectively. This result was also in conformity with the present research. In another study conducted by Adebayo *et al*., (2012) on microorganisms associated with spoilage of stored vegetables including cabbage in Uyo metropolis, Akwa-Ibom, Nigeria showed that *Escherichia coli* (28.6%) were the most predominant bacterial isolates associated with vegetable spoilage in Uyo metropolis. This was followed by *Enterobacter* spp. (21.4%), *Staphylococcus aureus* (14.3%), *Erwinia* spp.

(14.3%) *and Pseudomonas* spp. (14.3%) while *Salmonella* spp. (7.1%) was least predominant. This result is contrary to the present study due to present of *Erwinia* and *Enterobacter*.

CONLUSIONAND RECOMMENDATION

Conclusively, the results of the present study have revealed the spoiled cabbages are mainly contaminated with *Bacillus cereus, Staphylococcus aureus, Salmonella spp, Escherichia coli Klebsiella and Pseudomonas*. The fungal isolates are *Rhizopus spp, Aspergillus spp*, and *Penicillium spp*. Presence of these bacteria and fungi on cabbage, most especially Coliforms and *Aspergillus spp* poses a serious threat to health of consumers as the organism especially *Aspergillus* could produce mycotoxins, which are lethal when consumed which poses a serious threat to health of consumer and sellers of cabbage at Sabon-gari market Kano. It is therefore necessary and important that both the farmers and sellers to take necessary and appropriate precautions in preventing contamination and eating of contaminated vegetables. This will however reduce the risk of mycotoxins associated with fungi contamination which are deleterious to human health.

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