Bacteriological Assessment of Lettuce Vended in Benin City Edo State, Nigeria

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***Abstract*—** *The microbiological content of Lettuce (a vegetable), commonly vended in the Benin metropolis of Edo state were evaluated. Five vending locations were chosen for the study. Whole and soft rot samples were purchased and analysed for microbiological composition. Results showed high counts in soft rot samples in lettuce. Nutrient agar plated lettuce samples had bacterial counts in the range of 2.0x 103 to 4.7x10^7. Pseudomonas species was the dominant species found in lettuce samples. Bacillus species was isolated from one location in the lettuce samples. Mac Conkey agar plated lettuce plated had bacterial counts in the range of 2.3 x 10^3 to 5.7x 10^7. Enterobacter species, E. coli, and Klebsiella species were the dominant species isolated. Though, Proteus species was isolated from lettuce samples obtained from location five only. The study observes that consuming soft rot samples could pose a risk of introducing pathogens to the consumer due to their high microbial counts and could be detrimental to the health of the consumer.*

***Keywords*— Bacteriological Assessment, Lettuce, Benin City, microbiological content.**

I. INTRODUCTION

Food safety is of growing concern for consumer and professionals in the food industry worldwide. Food safety in ready to eat produce especially raw foods live fruits and vegetables has long been an object of study with many assessing the microbiological condition of raw fresh vegetables available in street markets as well as in self service and fast food restaurants (Angela *et al*., 2010).

Fresh vegetables are commonly found vended on the streets and in shops under both hygienic and unhygienic conditions. While many are less concerned with the processing and hygiene of these vegetables for consumption, they pose a direct risk of causing microbial food borne illness particularly when highly contaminated with microorganisms. Micronutrients, vitamins and fibre for humans can be easily metabolized from ingested vegetables which are known to be an extraordinary dietary source of nutrients, and are thus vital for health and well being. Well balanced diets, rich in vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2006).

Normal microbial flora characteristic of living organisms are also found in fruits and vegetables which may be altered while transporting from farm to the table (Margaret *et al*., 2009). Differences in microbial profiles of various vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007; Ofor *et al*., 2009).

Vegetables may also be contaminated whilst growing in fields or during the stages of harvesting, processing, distribution, sale and use. The lack of effective antimicrobial treatments at any step from planting to consumption means that pathogens introduced at any point may be present on the final food product. Even when available antimicrobials are applied, they may bring about a change in the final product. Such changes may include a change in the taste, colour, or the quality of the product. Fresh vegetables may be washed or treated specifically to minimize microbial load (FDA, 2000). As much as possible vegetables should be purchased from known sources or from sources known to operate standard hygienic practices while the purchase of these food materials from streets and open markets should be avoided. This is because the common practice of cooking some vegetables particularly leaves half cooked does not allow for the total elimination of microbial pathogens, while other vegetables may be eaten fresh without cooking as in the case of salad, thus directly exposing the digestive system to the threat of these pathogens.

The objectives of this study therefore were to evaluate the bacteriologic assessment of lettuce from street vended locations in Benin city Edo state and to identify the bacteria genus present on locally obtained lettuce.

II. MATERIALS AND METHODS

2.1 Lettuce Samples

Lettuce a vegetable were used for this study representing a commonly consumed vegetable in Nigeria. A total of 100 samples of lettuce were purchased from 5 different vending locations in Benin metropolis in Edo State. The vegetable from each sampling location were purchased and transported to the laboratory in a cool box at $\pm 4^0$c.

2.2 Preparation of Samples for Microbiological Analysis

Ten grams of lettuce were collected individually using a sterile scapel. These were separately added to 90ml of 0.1percent, peptone water and homogenized separately in a blender. One millilitre of each homogenate was transferred to separate test tubes containing 9ml peptone water to obtain a dilution of 10^{-1}. In a similar manner, 1ml each was transferred from this dilution to separate test tubes containing 9ml diluents and the process was repeated until a dilution of 10^{-9} was obtained for the lettuce samples.

2.3 Enumeration of Micro Organisms

0.1ml from each dilution of samples was transferred to plates of nutrient agar using the spread plate technique. Plates containing nutrient agar were incubated at 37^0C for 18-24hrs. Counts were made after incubation from plates having 30-300 colonies.

2.4 Identification of Bacterial Isolates

Bacterial colonies with characteristic edges, colours and sizes were isolated and purified by subculturing on nutrient agar plates and examined with a hand lens and each isolate subjected to biochemical test using the Bergey's manual of systematic bacteriology. The different tests carried out were used in identifying the isolates to their genus level.

III. RESULTS

Microbiological analyses of both whole and soft rot lettuce samples revealed that soft rot samples had the highest bacterial counts as compared to the whole samples. Soft rot samples had higher bacterial counts than whole samples as shown in table 7 and 11.The total viable count of soft rot lettuce samples were in the range of $2.1x\ 10^7$ to $5.7x10^7$cfu/g while whole samples had its total viable count as $2.0x10^3$ to $6.4x10^3$cfu/g.

Lettuce samples plated on nutrient agar revealed that *pseudomonasspecies* was the dominant organism found in both whole and soft rot samples obtained from locations 1 to 5. *Bacillusspecies* was isolated from soft rot samples obtained at location 5 only.A total number of six genera of microorganisms were isolated from lettuce samples which include *Pseudomonas spp* (23%), *Bacillus spp*(4%), *Enterobacterspp* (23%), *Klebsiellaspp* (23%), *Escherichia coli* (23%) and *Proteus spp* (4%).

Morphological characteristics of the test organisms revealed that the diameter of the colonies were in the range of 0.2-3.0mm.

TABLE 1
MORPHOLOGICAL CHARACTERISTICS OF THE ISOLATE

Sample code	Organism	Colony Characteristics
L1.	Pseudomonas sp	Greenish colonies of 0.4mm in diameter, circular, raised, opaque, with entire edges.
L 2.	Escherichia coli	Pink, convex, opaque, smooth surface, entire edge, circular, 1-2mm in diameter
L 3.	Proteus sp	Milky, convex, opaque, smooth surface, mucoid, spreading 2-3mm in diameter.
L4.	Bacillus sp	Creamish colonies of 0.5mm in diameter, irregular, flat, opaque with curled edges.
L 5.	Klebsiellasp	Pink, convex, opaque, smooth surface, circular, entire edge, 1-2mm in diameter
L 6.	Enterobactersp	Colourless, flat, serrated edge circular, 1-2mm in diameter.

Key: L – Lettuce

TABLE 2
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 1

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-01	4.1×10^3	*Pseudomonas sp*	LSN1-01	3.9×10^7	*Pseudomonas sp*
LWN2-01	3.2×10^3	*Pseudomonas sp*	LSN2-01	3.7×10^7	*Pseudomonas sp*
LWN3-01	3.3×10^3	*Pseudomonas sp*	LSN3-01	4.2×10^7	*Pseudomonas sp*
LWN4-01	2.0×10^3	*Pseudomonas sp*	LSN4-01	4.4×10^7	*Pseudomonas sp*
LWN5-01	2.7×10^3	*Pseudomonas sp*	LSN5-01	4.5×10^7	*Pseudomonas sp*
LWN6-01	2.3×10^3	*Pseudomonas sp*	LSN6-01	4.7×10^7	*Pseudomonas sp*

TABLE 3
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 2

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-02	2.0×10^3	*Pseudomonas sp*	LSN1-02	3.7×10^7	*Pseudomonas sp*
LWN2-02	2.1×10^3	*Pseudomonas sp*	LSN2-02	3.5×10^7	*Pseudomonas sp*
LWN3-02	2.6×10^3	*Pseudomonas sp*	LSN3-02	3.7×10^7	*Pseudomonas sp*
LWN4-02	3.1×10^3	*Pseudomonas sp*	LSN4-02	3.6×10^7	*Pseudomonas sp*
LWN5-02	2.5×10^3	*Pseudomonas sp*	LSN5-02	3.9×10^7	*Pseudomonas sp*
LWN6-02	5.7×10^3	*Pseudomonas sp*	LSN6-02	2.1×10^7	*Pseudomonas sp*

TABLE 4
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 3

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-03	3.9×10^3	*Pseudomonas sp*	LSN1-03	3.1×10^7	*Pseudomonas sp*
LWN2-03	3.7×10^3	*Pseudomonas sp*	LSN2-03	2.9×10^7	*Pseudomonas sp*
LWN3-03	2.0×10^3	*Pseudomonas sp*	LSN3-03	3.7×10^7	*Pseudomonas sp*
LWN4-03	4.3×10^3	*Pseudomonas sp*	LSN4-03	3.3×10^7	*Pseudomonas sp*
LWN5-03	2.6×10^3	*Pseudomonas sp*	LSN5-03	3.5×10^7	*Pseudomonas sp*
LWN6-03	2.7×10^3	*Pseudomonas sp*	LSN6-03	4.1×10^7	*Pseudomonas sp*

TABLE 5
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 4

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-04	4.3×10^3	*Pseudomonas sp*	LSN1-04	2.1×10^7	*Pseudomonas sp*
LWN2-04	3.5×10^3	*Pseudomonas sp*	LSN2-04	3.2×10^7	*Pseudomonas sp*
LWN3-04	3.2×10^3	*Pseudomonas sp*	LSN3-04	3.1×10^7	*Pseudomonas sp*
LWN4-04	2.1×10^3	*Pseudomonas sp*	LSN4-04	3.3×10^7	*Pseudomonas sp*
LWN5-04	2.3×10^3	*Pseudomonas sp*	LSN5-04	3.2×10^7	*Pseudomonas sp*
LWN6-04	3.7×10^3	*Pseudomonas sp*	LSN6-04	3.1×10^7	*Pseudomonas sp*

TABLE 6
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 5

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-05	2.1×10^3	*Pseudomonas sp*	LSN1-05	3.0×10^7	*Pseudomonas sp, Bacillus sp*
LWN2-05	2.3×10^3	*Pseudomonas sp*	LSN2-05	3.1×10^7	*Pseudomonas sp, Bacillus sp*
LWN3-05	2.9×10^3	*Pseudomonas sp*	LSN3-05	3.2×10^7	*Pseudomonas sp, Bacillus sp*
LWN4-05	3.5×10^3	*Pseudomonas sp*	LSN4-05	2.1×10^7	*Pseudomonas sp, Bacillus sp*
LWN5-05	2.7×10^3	*Pseudomonas sp*	LSN5-05	3.5×10^7	*Pseudomonas sp, Bacillus sp*
LWN6-05	3.4×10^3	*Pseudomonas sp*	LSN6-05	3.7×10^7	*Pseudomonas sp, Bacillus sp*

TABLE 7
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 1

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWM1-01	2.4×10^3	*Escherichia coli*	LSM1-01	4.4×10^6	*Enterobacter sp, klebsiella sp, E. coli*
LWM2-01	2.3×10^3	*Escherichia coli*	LSM2-01	5.4×10^6	*Enterobacter sp, klebsiella sp, E. Coli*
LWM3-01	2.6×10^3	*Escherichia coli*	LSM3-01	5.7×10^6	*Enterobacter sp, klebsiella sp, E. Coli*
LWM4-01	3.1×10^3	*Escherichia coli*	LSM4-01	4.3×10^6	*Enterobacter sp, klebsiella sp, E. Coli*
LWM5-01	3.4×10^3	*Escherichia coli*	LSM5-01	4.4×10^6	*Enterobacter sp, klebsiella sp, E. Coli*
LWM6-01	3.2×10^3	*Escherichia coli*	LSM6-01	4.7×10^6	*Enterobacter sp, klebsiella sp, E. Coli*

TABLE 8
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 2

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWM1-02	5.2×10^3	*Escherichia coli*	LSM1-02	5.4×10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM2-02	6.4×10^3	*Escherichia coli*	LSM2-02	5.6×10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM3-02	2.2×10^3	*Escherichia coli*	LSM3-02	5.4×10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM4-02	4.1×10^3	*Escherichia coli*	LSM4-02	5.5×10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM5-02	3.4×10^3	*Escherichia coli*	LSM5-02	5.3×10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM6-02	4.2×10^3	*Escherichia coli*	LSM6-02	5.2×10^7	*Enterobacter sp, klebsiella sp, E. Coli*

TABLE 9
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 3

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWM1-03	3.7×10^3	*Escherichia coli*	LSM1-03	$4.5X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM2-03	2.3×10^3	*Escherichia coli*	LSM2-03	$4.4X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM3-03	3.5×10^3	*Escherichia coli*	LSM3-03	$5.2X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM4-03	2.4×10^3	*Escherichia coli*	LSM4-03	$5.3X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM5-03	3.4×10^3	*Escherichia coli*	LSM5-03	$5.4X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM6-03	2.4×10^3	*Escherichia coli*	LSM6-03	$4.9X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*

TABLE 10
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 4

Sample code	Whole samples	*Organism found*	Sample code	Soft rot samples	Organism found
LWM1-04	2.3×10^3	*Escherichia coli*	LSM1-04	$4.5X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM2-04	$2.6x\ 10^3$	*Escherichia coli*	LSM2-04	$4.2X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM3-04	$2.6x\ 10^3$	*Escherichia coli*	LSM3-04	$4.1X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM4-04	$2.4x\ 10^3$	*Escherichia coli*	LSM4-04	$4.0X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM5-04	$2.1x\ 10^3$	*Escherichia coli*	LSM5-04	$5.0X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM6-04	$2.5x\ 10^3$	*Escherichia coli*	LSM6-04	$5.1X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*

TABLE 11
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 5

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWM1-05	$4.2x\ 10^3$	*Escherichia coli*	LSM1-05	$4.1X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM2-05	$4.5x\ 10^3$	*Escherichia coli*	LSM2-05	$4.4X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM3-05	$6.4x\ 10^3$	*Escherichia coli*	LSM3-05	$4.3X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM4-05	$4.6x\ 10^3$	*Escherichia coli*	LSM4-05	$5.1X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM5-05	$5.3x\ 10^3$	*Escherichia coli*	LSM5-05	$5.7X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM6-05	$5.7x\ 10^3$	*Escherichia coli*	LSM6-05	$5.4X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*

KEY:
LSN 1-6 LETTUCE SOFT ROT SAMPLES PLATED ON NUTRIENT AGAR
LWN 1-6 LETTUCE WHOLE SAMPLES PLATED ON NUTRIENT AGAR
LWM 1-6 LETTUCE WHOLE SAMPLES PLATED ON MacConkey AGAR
LSM 1-6 LETTUCE SOFT ROT SAMPLES PLATED ON MacConkey AGAR
01 – 05 LOCATIONS FROM WHICH SAMPLES WERE PURCHASE

TABLE 12
BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES FROM LETTUCE

Isolate code	Grams reaction	Cell morphology	Oxidase	Catalase	Citrate	Starch hydrolyses	Spore test	H_2S	MR	VP	Indole	Sucrose	Lactose	Motility	Maltose	Mannitol	Probable genera
	-	Rods	+	+	+	-	-	-	-	-	+	A/G	A/G	+	A	-	*Pseudomonas* sp
	-	Rods	-	+	-	-	-	-	+	-	+	A/G	-	+	-	A	*Escherischia coli*
	-	Rods	-	+	-	-	-	+	-	-	Neg	A/G	-	-	-	-	*Proteus* sp
	+	Rods	-	+	+	+	+	-	-	-	-	A/G	-	+	-	A	*Bacillus* sp
	-	Rods	-	+	+	-	-	-	-	+	-	A	-	-	A	A	*Klebsiellasp*
	-	Rods	-	+	+	-	+	-	+	+	-	A	A	+	-	A	*Enterobacter*sp

Note: +, Positive, -,Negative, A,acid production, G, gas production.

IV. DISCUSSION

There is an increasing consciousness of what people consume in the world today. This is because people tend to associate some food with health conditions after consumption or in later years of their life (Oriakpono, et *al*., 2011). This study evaluates the bacteriological quality of some vegetables sold in Benin metropolis, which were tagged locations 1,2,3,4 and 5 (representing the five market location).

Lettuce samples gotten from the five locations in Benin City had significant growth of microorganisms, but the microbial load of lettuce samples gotten from some locations where higher than the others, this may pose a threat to the health of regular consumers. Soft rot samples had higher bacterial counts than whole samples as shown in table 7 and 11.The total viable count of soft rot lettuce samples were in the range of $2.1x\ 10^7$ to $5.7x10^7$cfu/g while whole samples had its total viable count as $2.0x10^3$ to $6.4x10^3$cfu/g.

A total number of six genera of microorganisms were isolated from lettuce samples which include *Pseudomonas spp* (23%), *Bacillus spp*(4%), *Enterobacterspp* (23%), *Klebsiellaspp* (23%), *Escherichia coli* (23%) and *Proteus spp* (4%). The variation of microorganism isolated from lettuce may be due to the fact that lettuce is a creeping crop. The other possible reason for this variation may be due to harvesting, transportation, storage and during the vending process.

This study is in agreement with the work of (Brummel, 2006) which reports that soft rot is one of the significant spoilage diseases of vegetables. *Pseudomonas spp* have also been reported to cause spoilage of various vegetables like lettuce, spinach, tomato (Liao and Wells, 1987) which explains their high diversity. The soft rot group comprises several bacteria strains, of which *Pseudomonas spp* is a major soft rot causing bacteria (Toth *et al.*, 2001). *Pseudomonas spp* are unique among post harvest pathogens in that they are able to grow under refrigerated conditions and use a wide variety of compounds in samples as carbon which they utilize as energy sources. *Proteus* spp can cause serious disease condition on immune compromised patients causing infections of the respiratory tract (Jawetz *et al*., 1982). *Bacillus* spp is a gram negative spore forming bacteria, it is a well known food borne pathogen causing two types of illness: the emetic and the diarrheal syndrome this is due to the production of enterotoxins that can withstand harsh conditions. There were considerable growths of *Bacillus* spp in lettuce samples obtained from location 5 as shown in table 6. This agrees with the result obtained by Valero and co-workers as they isolated *Bacillus* spp from vegetables in ready to eat sandwiches and salad (Valero *et al*., 2002).

The vegetable (lettuce) have high water content or water activity this may encourage spoilage if not well preserved. The price of soft rot lettuce compared to whole samples is also a major factor encouraging the consumption of soft rot samples. This is because soft rot samples were found to be about half the price of whole samples in the market. Thus the people who purchase and consume the soft rot samples are at risk of the pathogen causing a disease.

V. CONCLUSION

Fresh vegetables are part of our daily diet. This study shows that there are a variety of organisms in both soft rot and whole samples of lettuce and these organisms may be introduced by various elements (wind, soil, water, insects, animals, human handling). They can become contaminated during growing, harvesting and transportation of the products. It is therefore necessary and important that both the farmer who harvests the vegetables into bags for transportation and the marketers take necessary and appropriate precautions in preventing contamination and eating of contaminated vegetables.

REFERENCES

[1] Angela O. E., Ibukunoluwa A. O., and Oranusi U. S. (2010) Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. *African Journal of Food Science Vol*. 4(5), pp. 291- 296, May 2010.

[2] Brummell, D.A. 2006. Cell wall disassembly in ripening fruit. *Functional Plant Biology*.33:103-119.

[3] Food and Drug Administration , (2000). Guide to minimize food safety hazards for fresh fruits and vegetables *www. Cfsanfda. Gov/html.*

[4] Jawetyz, E., J.L. Melnick, E.A. Adelberg (1982). Review of medical microbiology 15th edth. Lange med. Pub., Drawer L., Los Atlos, Califonia. 94022.pp.189-199.

[5] Kalia A, Gupta RP (2006). Fruit Microbiology, in Hui Y.H, J., Cano, M.P., Gusek, W.,Sidhu, J.W., Sinha, N.K. *Handbook of Fruit and Fruit processing. 1st Edition, Blackwell publishing*, pp 3-28.

[6] Liao, C.H., Wells, J.M. 1987. Diversity of pectolytic, fluorescent pseudomonads causing soft rots of fresh vegetables at produce markets. *Phytopathology* 77: 673-677.

[7] Margaret Barth, Thomas R. Hankinson, Hong Zhuang, and Frederick Breidt (2009). Microbiological Spoilage of Fruits and Vegetables *Compendium of the Microbiological Spoilage of Foods and Beverages*, Food Microbiology and Food Safety, DOI 10.1007/978-1-4419-0826-1_6.

[8] Ofor MO, Okorie VC, Ibeawuchi II, Ihejirika GO, Obilo OP, Dialoke SA (2009). Microbial Contaminants in Fresh Tomato Wash Water and Food Safety Considerations in South-Eastern Nigeria. Life Sci. J.,1:80-82.

[9] Oriakpono., Obemeata, Frank – PetersideNnenna and Ndome Christopher (2011). Microbiological assessment of stored *Tilapia guineensis African Journal of Food Science* Vol. 5(4), pp. 242 – 247,

[10] Ray B, Bhunia AK (2007). Fundamental Food Microbiology. 4th Edn.,*CRC Press, USA.* p. 492.

[11] Toth I.K., Avrova A.O., Hyman L.J., 2001. Rapid identification and differentiation of the soft rot erwinias by 16S-23S intergenic transcribed spacer-PCR and restriction fragment length polymorphism analyses. *Applied and Environmental Microbiology* 67: 4070-4076.

[12] Valero, M.L.A. Hernandez- Herrero, P.S. Fernandez, M.C. Saimeron (2002). Characterization of *Bacillus* isolated from fresh vegetables and minimally processed foods. *J. microbial.4:5-9.*