Research Journal of Applied Sciences, Engineering and Technology 3(5): 393-398, 2011
ISSN: 2040-7467

Received: February 03, 2011 Accepted: February 03, 2011 Published: May 25, 2011

Microbial Effects on Selected Stored Fruits and Vegetables under Ambient Conditions in Makurdi, Benue State, Nigeria

S.E. Obetta, T.U. Nwakonobi and O.A. Adikwu
Department of Agricultural and Environmental Engineering,
University of Agriculture, Makurdi, Nigeria

Abstract: Information on the level of contamination due to bacterial and fungal infestations on some of the freshly supplied fruits and vegetables in Makurdi, Benue State, Nigeria is lacking. This study investigated the microbial load of some freshly supplied fruits and vegetables in Makurdi which included Apples, Banana, Carrots and Oranges. As part of food quality surveillance study, this work looked at the incidence of pathogen associated with fruits and vegetables from three locations in Makurdi. The samples were evaluated for bacterial (coliforms and mesophiles) and fungal (mold and yeast) loads. Results of the microbial test showed that Apple had the average absolute highest microbial load with mesophilic aerobic plate count of 127 $\log_{10}$CFU/in^2, coliform, 71 $\log_{10}$CFU/in^2, yeast, 30 $\log_{10}$CFU/in^2 and mold, 22.3 $\log_{10}$CFU/in^2. Carrot and Banana followed with average absolute mesophilic aerobic plate count of 122.3 $\log_{10}$CFU/in^2 and 113 $\log_{10}$CFU/in^2, coliform of 97.7 and 42.7 $\log_{10}$CFU/in^2, yeast, 23.7 and 23.7 $\log_{10}$CFU/in^2 and mold, 20.7 and 28 $\log_{10}$CFU/in^2 respectively. Orange had the least microbial load with an average absolute mesophilic aerobic plate count of 27.3 $\log_{10}$CFU/in^2, coliform - 30.3 $\log_{10}$CFU/in^2, yeast - 21 $\log_{10}$CFU/in^2 and a mold count of 29.3 $\log_{10}$CFU/in^2. The ANOVA results showed highly significant effects ($p<0.01$) of bacteria, fungi and the fruit/vegetable types in the three locations. The interaction effects between the microbes and the fruit/vegetables were significant and increased with length of storage.

Key words: Bacteria, fruits/vegetables, fungi, microbial load, plate count

INTRODUCTION

Fruits and vegetables are horticultural products having living tissues with continuing metabolism, and thus subject to respiration, water loss and cell softening throughout the post harvest system. Surveys of raw fruits and vegetables demonstrate that there are potentials for wide range of these products becoming contaminated with microorganisms, especially the pathogenic ones (Kader, 1997). Since fruits and vegetables contribute greatly to human health and are often eaten raw (minimally processed products) there is need to ensure the safety of these produce by addressing common areas of concern in growing, harvesting, sorting, packing and distribution of fresh produce.

Many fruits and vegetable are rich sources of vitamins, such as vitamin C, folic acid (useful in synthesis of DNA), vitamin A, including minerals such as calcium and iron. They also contain dietary fibres which add bulk to intestinal content and useful in preventing constipation. Fruits and vegetables have similar nutritive properties; 70% of their weight is water, 3.5% protein and about 1% fat. They provide comparatively little energy. Consumption of fruits and vegetables can help achieve or maintain a healthy body weight (National Institute of Research on Food and Nutrition Rome, Italy, 1998). Fresh produce may contain plant pathogenic and spoilage microorganisms which could reduce shelf-life and acceptability of fresh produce. Microbes responsible for fruits and vegetable spoilage include bacteria (*Lactobacillus* spp.), yeasts (*Saccharomycces* spp.) and molds (*Rhyzopus* spp.). These microbes render fresh fruits and vegetables unfit for human consumption; by causing their deterioration and leading to reduction in quality, texture, off flavour development and loss of nutrients. Microorganisms form part of the epiphytic flora of fruits and vegetables and many are likely to be present at the point of consumption. Majority of bacteria found on the surface of plants are usually the Gram-negative which belong to the *Pseudomonas* spp. or to the enterobacteraceae (Lund, 1992). The range of microorganisms associated with spoilage or contamination of fruits and vegetables include; bacteria, parasites, protozoa and viruses and these are often associated with contaminated water and/or food handlers (Beuchat, 1998). However, there are certain factors which contribute to the microbiological contamination arising from the consequence of treating soil with organic fertilizers such

Corresponding Author: S.E. Obetta, Department of Agricultural and Environmental Engineering, University of Agriculture, Makurdi, Nigeria

as manure and sewage sludge and from irrigation water (Ward and Irving, 1987). In addition, the application of technologies such as cutting, slicing, skinning, bruising and shredding that removes the natural protective barriers of the intact plant thereby creating openings for the entry of the contaminant microorganisms.

Nigeria produces a variety of tropical and sub-tropical fruits and vegetables mainly for domestic consumption. Among some important fruits are banana, citrus, mangoes and pineapples with the principal vegetables as tomatoes and onions. Present awareness on the importance of routine fruit and vegetable consumption and the attendant increase in the urgent need for the produce and has placed a substantial demand for all-year-round availability of the produce. However, inadequate availability of appropriate storage facilities has continued to militate against the availability and sufficiency of the produce in Nigeria. This is because storage of harvested products under controlled conditions (controlled temperature and relative humidity) is known to retard the growth of post harvest spoilage and pathogenic microorganisms (Nguyen and Cardin, 1994). Although washing with a wide range of available disinfecting agents can reduce microbial load, none is able to eliminate the contaminating pathogens. Given much scope for control lies in post harvest management of the two most important determinants of storage life and quality; respiration and transpiration. Both need to be limited but not stopped and proper control of temperature and relative humidity and sanitation (good management practices and good agricultural practices to reduce or eliminate microbial pathogens on fresh fruits and vegetables) are keys to maximizing storage life and marketable quality.

Optimum relative humidity for storage of fresh fruits and vegetables lies between 85-90% (Aharoni, 2004). The rate of respiration doubles for every 10ºC rise in temperature (Fallik and Aharoni, 2004). It is of great importance to note that factor such as variety and pre-harvest conditions determine the physiological response to storage environment.

About two thirds of the spoilage of fruits and vegetables is caused by molds. Members of the genera *Penicillium, Aspergillus, Botrytis* and *Ryzopus* are commonly involved in this process. The spoilage is usually associated with cellulytic or pectinolytic activity, which causes softening and weakening of plant structures (Garg *et al*., 1990; Bracket, 1992). Exposing vegetables to various types of cutting has been shown to result in an increase in microbial numbers (Strauch, 1991; Francis *et al*., 1999).

This study is aimed at evaluating the effect of microbial loads on some fruits and vegetables in three locations in Makurdi after five-day storage at ambient environment.

MATERIALS AND METHODS

This study was conducted in the laboratory of the Department of Food Science and Technology, University of Agriculture, Makurdi, Benue State, Nigeria in 2009.

A set of three samples of four different fresh fruits and vegetables namely Apple, Orange, Carrot and Banana were bought randomly from three different locations (Wurukum, High-Level and Wadata) in Benue State giving a total of thirty six (36) items for the fruits/vegetables combination. These items were left in their natural environment as supplied and kept unwashed before the commencement of the experiments.

Microbial count: The pour plate technique was used to obtain the microbial count. Nine (9) mL of distilled water was measured using a pipette into each of the sterile test tubes and were sterilized in an autoclave for 15 min at 121ºC were brought out and allowed to cool to body temperature (24-25ºC). The fresh fruits and vegetables were cut into 1-2 cm pieces with a sterile scalpel and 1gm of the sample was mixed with 9 mL of distilled water and shaken properly.

Using a fresh pipette at each stage, a serial dilution was carried out to 10^{-4} dilution i.e. 1ml of the solution was drawn out using a pipette and was introduced into a second test-tube containing 9 mL of distilled water and this was done serially until the last tube with 10^{-4} dilution was attained. The Potato Dextrose Agar (PDA) was melted and cooled. Sterile petri dishes were set out for each dilution and they were labeled with the dilution number. 1ml of each dilution was pipetted into the centre of the appropriate dishes, using a fresh pipette for each dilution. Potato dextrose agar was poured into each of the plates, enough to cover the 1ml solution in them. The medium was allowed to solidify, then inoculated and incubated at 37ºC for 48 h. The numbers of colonies were counted with the use of a colony counter. The colony count will be calculated by multiplying the number of colonies counted by the reciprocal of the dilution and will be reported as colony count per area.

Fungal count: Some colonies were stripped off using a sterile wire loop and placed on grease free glass slide. This set-up was stained using lacto phenol cotton blue, rinsed with sterile water and allowed to dry. A compound microscope with a magnification of 100 was used to view the fungi morphologies and results were compared with standard charts which aided the identification. The set up was monitored for five days, specifically for day 1, day 3 and day 5 in the Microbiology Laboratory of the University of Agriculture and results were generated.

Experimental design: The experimental design (Obi, 1995) for the statistical analysis followed a 2-

treatment effect (microbial population and fruits/vegetables) in a split-plot factorial design with Randomized Complete Block Design (RCBD) involving a 2-way classification with 3 observations (replications) per experimental unit. In one experimental unit, 2 factors (2 bacteria types) on each of the 4 fruits/vegetable were considered while in the other 2 fungi types (mold and yeast) on a set of same 4 fruits/vegetables were studied. Generated microbial population at the end of the experimentations for the different conditions and their interaction effects were subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

One general observation was that in each of the studies, the bacteria and fungi populations were varying in increment progressively from the Day-1, to Day- 3 and finally Day-5 of the study duration. Also observed conspicuously was that among the fruit/vegetable combinations, Orange recorded the least value in both bacterial and fungal counts.

Figure 1a and b showed the results of the absolute microbial population for both bacteria and fungi from Wurukum location after the 5 days duration of storage. For all the fruits and vegetable types, the absolute values of bacterial count were more in number than that of the fungal count. Of these values, orange recorded the least with a value of 39 $\log_{10}$CFU/in^2 for coliforms and 25 $\log_{10}$CFU/in^2 for mesophiles while the fungal population was put at 36 $\log_{10}$CFU/in^2 for mold and 19 $\log_{10}$ CFU/in^2 for yeast. These observed values are attributable to the acid content of the orange cover which tends to offer it protection against most micro-organisms. Carrot was heavily loaded with the micro-organisms for its sugar content at both instances of bacteria and fungi status, followed by Apple and Banana in that order of decreasing magnitude. The mesophilic population showed higher values than the coliform infections while in the fungal effect; the mold values appeared greater in the aggregate terms. Table 1 the summary the Analysis of Variance (ANOVA) at the Wurukum location. On this table it was observed that both bacterial and fungal effect of the storage stability of showed the fruits/vegetables are highly significant ($p<0.01$) but there was no statistical interaction between the fungal type and the fruit/vegetable on Day-1. However, the interaction between the bacteria type and the fruit/vegetable showed significance at 5% probability. This trend was observed also for Day-3 while interaction was significant ($p<0.01$) in both cases for Day-5.

Figure 2a and b showed the results of the absolute microbial population representing the 5 days duration of the storage in the High Level location of the study areas for both bacteria and fungi populations. It was observed that number or colonies of mesophilic

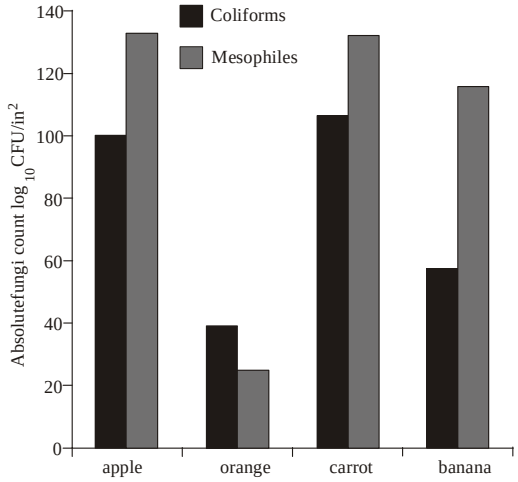

Fig. 1a: Bacteria population in Wurukum location

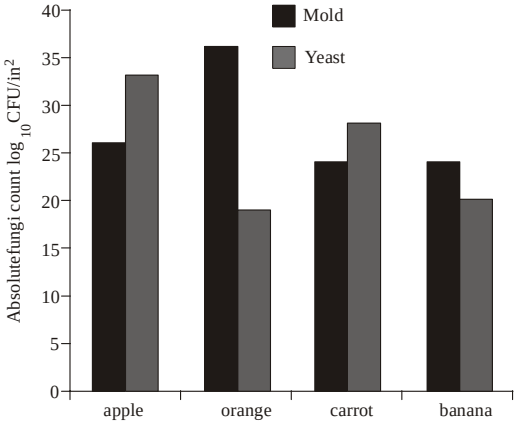

Fig. 1b: Fungi population in Wurukum location

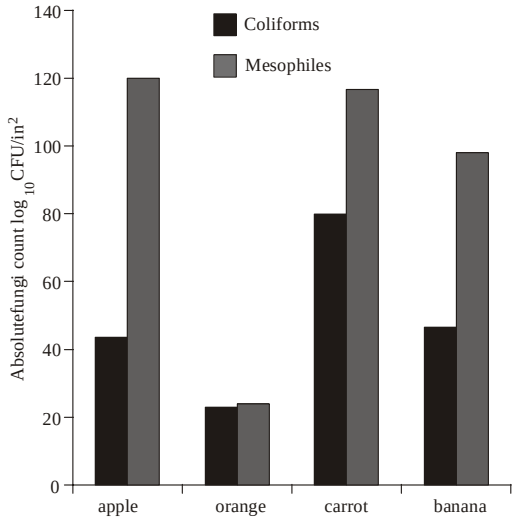

Fig. 2a: Bacteria population in high level location

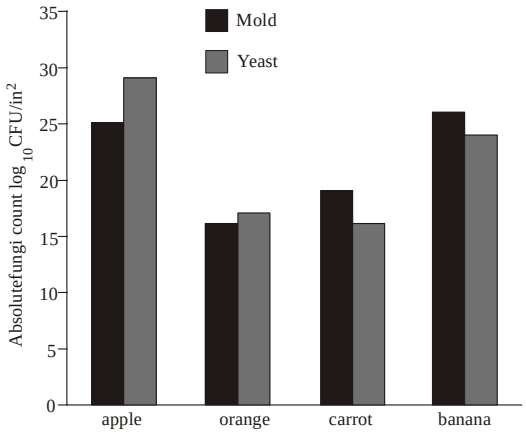

Fig. 2b: Fungi population in high level location

Table 1: Summary of analysis of variance for the effect of microbial population on fruits and vegetables at Wurukum location

Day -1			
Source	df	MS	F-ratio
Bacteria	1	660.68	3450.46**
Fruit/Vegetable	3	313.09	5.65*
Interaction	3	242.58	4.38*
Fungi	1	1.5504	534.62**
Fruit/Vegetable	3	2.7215	331.89**
Interaction	3	9.0015	1.098^{NS}
Day-3			
Bacteria	1	665.707	14254.96**
Fruit/Vegetable	3	1100.67	19.84**
Interaction	3	246.65	4.44*
Fungi	1	6.615	170**
Fruit/Vegetable	3	17.805	1005.93**
Interaction	3	16.143	912.03**
Day-5			
Bacteria	1	150.5004	3609.12**
Fruit/Vegetable	3	2376.21	163.09**
Interaction	3	181.4	12.45**
Fungi	1	12.76	387.84**
Fruit/Vegetable	3	13.17	9.696**
Interaction	3	40.167	29.57**

$F_{1,12,0.05} = 4.75$; $F_{3,12,0.05} = 3.49$; $F_{1,12,0.01} = 9.33$; $F_{3,12,0.01} = 5.95$; NS: Not significant; MS: Mean square; *: Significant at $p \leq 0.05$; **: Significant at $p \leq 0.01$

bacteria (low temperature loving bacteria) were highest (120 $\log_{10}$CFU/in^2) on Apple, followed by Carrot (117 $\log_{10}$CFU/in^2), Banana (98 $\log_{10}$CFU/in^2) and Orange (24 $\log_{10}$CFU/in^2) in that order of decreasing magnitude. The trend of the occurrence of coliforms however, varied in magnitude with Carrot having the highest number of (80 CFU/in^2) followed by Banana with (47 $\log_{10}$CFU/in^2), Apple (44 $\log_{10}$CFU/in^2) and then Orange with the least value of 23 $\log_{10}$CFU/in^2 count. The fungal population however, ranged from 24 $\log_{10}$CFU/in^2 to29 $\log_{10}$CFU/in^2 for the yeast/mold for Apple and Banana while the values ranged from 16 $\log_{10}$CFU/in^2 to19 $\log_{10}$CFU/in^2 for the same yeast/mold for Carrot and Orange. In Table 2, it

Table 2: Summary of analysis of variance (ANOVA) for the effect of Microbial population on fruits and vegetables at high level location

Day -1			
Source	df	MS	F-ratio
Bacteria	1	488.304	55489.07**
Fruit/Vegetable	3	406.837	10.10**
Interaction	3	124.767	3.10^{NS}
Fungi	1	3.5267	172.88**
Fruit/Vegetable	3	5.8833	19.12**
Interaction	3	2.3922	7.78**
Day-3			
Bacteria	1	1264.402	190.135**
Fruit/Vegetable	3	723.94	6.87**
Interaction	3	159.62	1.51^{NS}
Fungi	1	0.001667	0.57^{NS}
Fruit/Vegetable	3	20	1333.33**
Interaction	3	1.81	120.67**
Day-5			
Bacteria	1	1006.215	114996**
Fruit/Vegetable	3	1297.07	15.465**
Interaction	3	138.55	1.65^{NS}
Fungi	1	5.9	907.69**
Fruit/Vegetable	3	257.87	505.62**
Interaction	3	7.135	13.99**

$F_{1,12,0.05} = 4.75$; $F_{3,12,0.05} = 3.49$; $F_{1,12,0.01} = 9.33$; $F_{3,12,0.01} = 5.95$; NS = Not significant; MS: Mean square; **: Significant at $p \leq 0.01$

was observed that the effect of bacterial and fungal organisms on the fruits/vegetables for high-level were highly significant ($p<0.01$) for Day-1 and Day-5 although fungal effect on Day-3 was not significant while that for bacteria was highly significant at $p<0.01$on the same day. There was no statistical significance on the interaction between the bacterial type effect and the fruit/vegetable on all the days studied, while the fungal and fruit/vegetable interactions were very significant at 1% probability for all the days of the studies.

The results on the Wadata location were shown in Table 3 that the Bacteria, fruit/vegetable factors were highly significant ($p<0.01$) on Day-1, Day-3 and Day-5. The individual fungi and fruit/vegetable effects were also significant ($p<0.01$) for Day-1 and Day-5. It was observed however that fungal effect was not significant on Day-3 just as the interaction of bacteria and fruit/vegetable combinations, while only fruit/vegetable factor was highly significant ($p<0.01$) as well as the interaction of fungi and fruit/vegetable factors. On Day-5, fruit/vegetable interaction was not significant but fruit/vegetable effect was highly significant ($p<0.01$). Fungi effect was highly significant, so also was fruit/vegetable and their interaction all very significant at $p<0.01$. On Fig. 3a and b are the indications of the results of the absolute microbial population spanning the 5 days duration of the storage at Wadata study location for both bacteria and fungi populations. It was observed from the results that mesophilic bacteria had the greatest colony count (133 $\log_{10}$CFU/in^2) for the Apple, followed by that of Banana (125 $\log_{10}$CFU/in^2), Carrot (118 $\log_{10}$CFU/in^2) and Orange

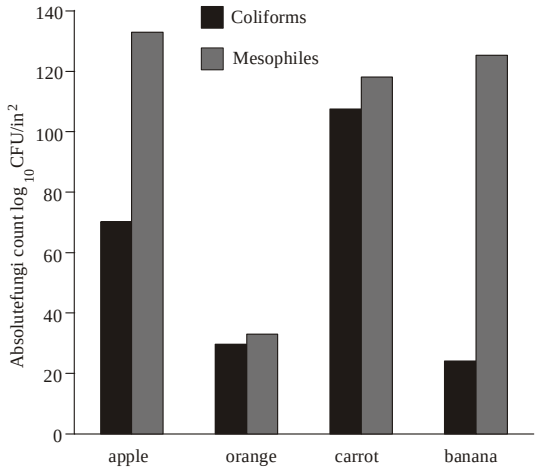

Fig. 3a: Bacteria population in Wadata location

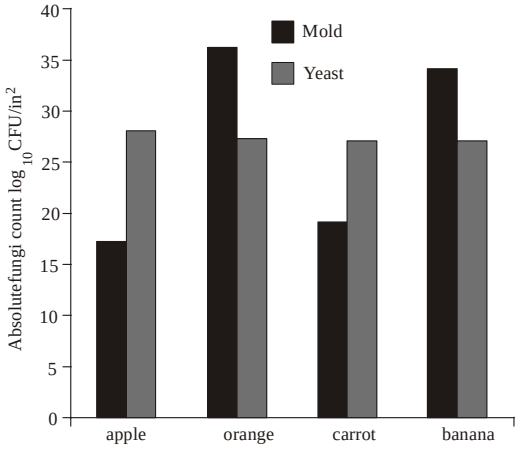

Fig. 3b: Fungi population in Wadata location

(33 $\log_{10}$CFU/in^2) in that trend. The order of the occurrence of coliforms however, varied in magnitude with Carrot having the highest number of (107 $\log_{10}$CFU/in^2), Apple had (70 $\log_{10}$CFU/in^2), followed by Orange with 29 $\log_{10}$CFU/in^2 and then Banana having the least value (24 $\log_{10}$CFU/in^2), of the count. The fungal population was in the range of 19 to36 $\log_{10}$CFU/in^2 for the same yeast/mold count between Carrot and Orange while the values ranged from 27 to 34 $\log_{10}$CFU/in^2 for the yeast /mold count between Apple and Banana.

Towards the end period of this study, there was noticeable evidence of shrinkage on the physical structure of these farm produce. This shows that natural environment does not provide worthwhile protection not only of the appearance but exposure to microbiological infestation. Some measures can reduce the microbial contamination of fruits and vegetables. Organic acids alone or in combination with chlorine have shown in experimental design to effectively reduce microbial load

Table 3: Summary of analysis of variance (ANOVA) for the effect of microbial population on fruits and vegetables at Wadata location

Day -1			
Source	df	MS	F-ratio
Bacteria	1	483.304	54920**
Fruit/Vegetable	3	406.837	10.1**
Interaction	3	124.77	3.1^{NS}
Fungi	1	3.527	172.05**
Fruit/Vegetable	3	5.884	19.1**
Interaction	3	2.392	7.77**
Day-3			
Bacteria	1	1362.03	62766**
Fruit/Vegetable	3	769.98	6.78**
Interaction	3	313.71	2.76^{NS}
Fungi	1	0.327	6.996^{NS}
Fruit/Vegetable	3	10.45	14.43**
Interaction	3	16.2	349**
Day-5			
Bacteria	1	2573.01	$15.59x10^{6}$**
Fruit/Vegetable	3	1677.44	7.82**
Interaction	3	746.11	3.48^{NS}
Fungi	1	0.54	432**
Fruit/Vegetable	3	36.18	616.33**
Interaction	3	57.83	963.83**

$F_{1,12,0.05} = 4.75$; $F_{3,12,0.05} = 3.49$; $F_{1,12,0.01} = 9.33$; $F_{3,12,0.01} = 5.95$; NS: Not significant; MS: Mean square; **: Significant at $p \leq 0.01$

(Karapinar and Gonul, 1992; Dowe *et al*., 1997). Many studies suggest that hydrogen peroxide has potentials as sanitizing agent for fruits and vegetables (Beuchat, 1998). Potential exists for organic farming to contaminate fruits and vegetables with pathogens. Manure, bio-solids and irrigation water should be of a quality that does not introduce pathogens to the treated commodity. Modern technology of cold storage, CA storage, and use of refrigeration has alleviated the problems of fruit /vegetable infestations.

CONCLUSION

From this study, several factors but most prominently those under critical influence of propagating micro-organism proliferation in fresh fruits and vegetable were examined. Both fruits/vegetable and the type of pathogens are discovered to be exerting effects on the microbial load acquired during the period of study. Under the environment of this study, Apple had the average absolute highest aerobic plate count of 127 $\log_{10}$CFU/in^2 for the bacterial population while in the fungi load, the same Apple yielded yeast count of 30 $\log_{10}$CFU/in^2. On the other hand, Orange fruit appeared to have had the least microbial load, having an average absolute mesophilic aerobic plate count of 27.3 $\log_{10}$CFU/in^2 and fungi population of 21 $\log_{10}$CFU/in^2 for yeast and 29.3 $\log_{10}$CFU/in^2. The ANOVA study showed highly significant effects ($p<0.01$) of bacteria, fungi and the fruit/vegetable types in the three locations. Their interactions were found to be increasing in significance as

the storage days is increased. Much as these pathogen levels are not deleteriously harmful to human health, it is however a greater indication that much care should be taken in handling fruits and vegetables before consumption. Fruits and vegetables are to be sanitized, disinfected and washed thoroughly to be used further especially in fresh forms.

ACKNOWLEDGMENT

The efforts and individual contribution of each of the authors towards the sponsoring of this study are highly appreciated.

REFERENCES

Aharoni, N., 2004. Packaging modified atmosphere (MA) and controlled atmospehre (CA) principles and applications power point lecture slides. International research and developmetn course on post harvest biology and technology. The Volcani Centre, Isreal.

Beuchat, C.R., 1998. Surface decontamination of fresh fruits and vegetables eaten raw. A review. Food safety unit. World Health Organization, WHO/FSF/FOS/98-2.

Bracket, E.T., 1992. Shelf stability and safety of fresh Produce as Influenced by sanitation and disinfections. J. Food Prot., 10(55): 805-514.

Dowe, M.J., E.O. Jackson and J.G. Mori, 1997. Listeria monocytogenes survival in soil and incidence in agricultural soils. J. Food Prot., 60: 1201-1207.

Fallik, E. and Y. Aharoni, 2004. Postharvest physiology, pathology and handling of fresh produce, lecture notes. International research and development course on postharvest biology and technology. The Volcani Centre, Isreal, pp: 30.

Francis, G.A., C. Thomas and T. OBeirne, 1999. The microbiological safety of minimally processed vegetables. Intl. J. Food Sci. Technol., 34(1): 22-30.

Garg, N., J.J. Chuvey and D.F. Splift, 1990. Effects of processing conditions on the microflora of fresh cut vegetables. J. Food Prot., 53: 701-703.

Kader, A.A., 1997. Quality in Relation to Marketability of Fruits and Vegetables. Proceeding of the 5th Fruit Nut and Vegetable Eng. Symposium, University of Califonia, Davis. In: Studman, C.J. (Ed.), CIGR Handbook of Agricultural Engineering, ASAE, 1999, St Joseph MI, USA, 4(3): 243-272.

Karapinar, M. and S.A. Gonul, 1992. Removal of *Yersinia enterocolitica* from fresh parsley by washing with acetic acid or vinegar. Intl. J. Food. Microbiol., 16: 261 – 264.

Lund, B.M., 1992. Ecosystems in vegetable foods. J. Appl. Bact., 73(Suppl 21): 115S-135S.

Nguyen, C. and F. Cardin, 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food Sci. Nuts, 34: 371-401.

Strauch, D., 1991. Survival of micro organisms and parasites in excreta, manure and sewage sludge. Rev. Sc. Tech. off Intl. Epiz-10816-846.

Ward, E.F. and L.G. Irving, 1987. Virus survival on vegetables spray irrigated with waste water. Wat. Res., 21: 57-63.

Obi, I.U., 1995. Introduction to factorial experiments for agricultural, biological and social sciences research. 2nd Edn., Optimal Publishers, Enugu, Nigeria.

AUTHOR'S CONTRIBUTION

S.E. Obetta initiated the research topic, designed the experiment, and carried out the statistical analysis of the data. T.U. Nwakonobi co-supervised the research work. O.A. Adikwu collected the data.