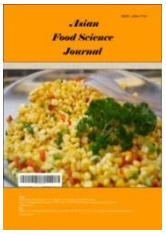

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Microbiological Efficacy of Hessian Bag on Leafy Vegetables during Distribution in (Aguleri) Anambra State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Vegetables are highly perishable commodities that easily get spoilt or deteriorate during handling along the supply chain from the producer to the final consumer. This study aims to compare the microbiological efficacy of hessian bags and non hessian bags on leafy vegetables (*Telfairia occidentalis*) Ugu and (*Ocimum gratissimum*) Nchuanwu leaves using hessian bags and non-hessian bagsduring distribution. Standard procedures on microbial count, isolationof microorganisms, purification of microbial isolates, biochemical identification of isolateswasused to identify the microorganisms present. The microbial load of the leafy vegetables from farmland to consumer increased progressively along the distribution chain. *Staphylococcus species* were the predominant bacteria in the leafy vegetables from the farmland. Other bacteria found were *Pseudomonas, Bacillus spp, Staphylococcus spp, Escherichia coli* and fungi *Penicillium spp, Aspergillus niger, Rhodotorula spp, Fusarium spp, Mucor spp.* The study showed that the microbial contents of the vegetables increased across the distribution line from farmland to consumer irrespective of the use of hessian bags.

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Keywords: Hessian bag; microbiological properties; leafy vegetables; distribution.

1. INTRODUCTION

Leafy vegetables are fresh and edible leaves of herbaceous plants which can be eaten raw or cooked [1]. Daily diet containing leafy vegetables have been strongly associated with overall good health, improvement of gastrointestinal health and vision, reduced risk for some forms of cancer, heart disease, stroke, diabetes, anaemia, gastric ulcer, rheumatoid arthritis, and other chronic diseases [2]. Vegetables are highly perishable commodities that easily get spoilt or deteriorate during handling along the supply chain from the producer to the finalconsumer. Deterioration and spoilage of fresh cut vegetables may be the result of biological, microbiological, physiological/biochemical or physical factors acting on them. These deterioration and losses decrease the quality of the vegetables.Vitamin C which a major micronutrient in vegetables begins to degrade immediately after harvest [3]. Loss of vitamin C is often used as an indicator of quality deterioration during postharvest handling including transportation, storage, and processing because it is highly susceptible to chemical and enzymatic oxidation and is highly water soluble [4]. Quantitative and qualitative losses of leafy vegetables mainly occur after harvesting, during transportation, processing and in storage [5].

The name "Hessian" is attributed to the historic use of the fabric as part of the uniform of soldiers from the former Landgraviate of Hesse and its successors, including the current German state of Hesse, who were called "Hessians" [6]. The hessian bag can help to prevent vegetables deterioration. It is the cheapest vegetable fibre procured from the bast or skin of the plant's stem and the second most important natural vegetable fibre after cotton, in terms of usage, global consumption, production, and availability. It is completely biodegradable, has high tensile strength, low extensibility, and ensures better breath ability of fabrics. Jute has been used since ancient times in Africa and Asia to provide cordage and weaving fibre from the stem and food from the leaves. Non- hessian bags includes bags such as polythene bag, nylon bags, nylon sack and plastic bags that can be used for transportation of leafy vegetables. Leafy vegetables can be preserved through sun drying which is the logical for rural households that have limited resources because of the low cost of such preservation. Other methods include, solar-drying, freezing, canning or bottling [7]. Indicates that the commonly practiced preservation methods traditionally are boiling and sun drying.

A short supply chain are few passages from the raw materials to the final consumer, all mainly confined in the local markets are increasingly common in food sector although still represents a niche [8]. Vegetable distribution majorly involves movement from farmland where they are cultivated to the distributor who moves them to strategic places(market) while retailers pick them at larger quantities and sell to the final consumers. The use of hessian bags as a type of jute bag in preservation of vegetables during post-harvest operations is a more reliable method because hessian ability to allow the contents of bags to breathe makes it excellent for preventing or minimizing rotting due to trapped moisture. In some cases, hessian bag can even be specially treated by washing the bag using 2% of detergent solution and distilled water and finally dried in a vaccum oven at 70^0C [9]. This avoids specific kinds of rot such as block rot, soft rot and decay on the leafy vegetables caused by some species of bacteria (*Pseudomonas, Bacillus spp, Staphylococcus spp, Escherichia coli*) and fungi (*Penicillium spp, Aspergillus niger, Rhodotorula spp, Fusarium spp, Mucor spp*). This study aims to compare the use of hessian bags and non-hessian bagsin the microbiological properties of leafy vegetables during distribution in (Aguleri) Anambra State located in the south-eastern part of Nigeria.

2. MATERIALS AND METHODS

2.1 Source of Materials

The media and reagents used in this research work were of good standards and specification and were prepared according to the manufacturer instruction.The vegetables samples (*Telfairia occidentalis*) Ugu and (*Ocimum gratissimum*) Nchuanwu used in this study were collected from Aguleri,Anambra State,Nigeria during the distribution of leafy vegetables in the supply chain from farmland (control), distributors, retailer and to the consumer on the same day using sterilized hessian bags and non- hessian bags (nylon sack) and were transported to the laboratory.

2.2 Study Area

The experiment was carried out in the Department of Applied Microbiology and Brewing, NnamdiAzikiwe University, Awka.

2.3 Determination of the Changes in the Microbial Properties of the Leafy Vegetables during Distribution

2.3.1 Isolation of microorganisms from the leafy vegetables

20g of the vegetables samples were measured and finely chopped using a sterile knife and grinded using a sterile mortar and pestle. The extract was obtained by adding 50ml of distilled water. 1ml of the extract was subjected to a ten-fold dilution series. 1ml was pipetted from dilution tube (10^{-4}) into sterile petri dishes. Nutrient agar, MacConkey agar, Sabouraud dextrose agar were prepared accordingly to the manufacturer instructions and were sterilized using autoclave at 121^0C for 15 mins cooled and poured into each of the plates. Nutrient agar plates, MacConkey agar plates and Sabouraud dextrose agar plates and the plates were incubated at 37^0C for 24 hr. The colonies on the Nutrient agar, MacConkey agar and Sabouraud dextrose agar were observed counted and recorded [10]. This was done for leafy vegetables samples from the hessian bag and non-hessian bag.

2.3.2 Purification of the Microbial Isolates from the leafy vegetables

The bacteria and fungi isolate which developed on the plates were randomly picked and purified by sub culturing on Nutrient agar plates before transferring to Nutrient agar slants. The fungal isolates were purified using the same method as the bacterial isolates but they were Sub cultured into Sabouraud dextrose agar plates before transferring into the Sabourand slants. These isolates were stored at 4^0C in the refrigerator as stock cultures for characterization and then further identification was done through biochemical tests [11].

2.4 Biochemical Identification of Isolates

This test was carried out for the complete identification of bacteria isolates from specimen with significant growth.

2.4.1 Gram staining

Gram staining technique was carried out to determine whether the isolates were able to retain the basic dye or the counter stain within their cell wall. A discrete colony was picked from the pure culture and smeared with normal saline on a clean grease free slide. The smear was allowed to air dry and heat fixed by passing the slide three times over a flamed Bunsen burner. After heat fixing, the smear was covered with crystal violet for 1 min and rapidly rinsed with clean water. After rinsing, the smear was covered with lugol's iodine for 30-60 sec and then rinsed with clean water. It was decolourized with acetone and rinsed immediately with clean water. The smear was finally counter stained with secondary dye (saffranin) for 2 mins and rinsed with clean water. The slide was placed on the staining rack to air dry. After air drying, a drop of immersion oil was placed on the smear and examined microscopically using x100 objective lens [12].

2.4.2 Catalase test

This demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H_2O_2). A single colony was taken on clean glass slide using a sterile wire loop. The production of gas bubbles on the addition of 0.5 ml of hydrogen peroxide indicated a positive reaction [13].

2.4.3 Simmon's citrate test

This test is used to check the ability to utilize citrate as its carbon and energy source [14]. The medium was poured in two test tubes and kept in slant condition, when cooled a single pure isolated colony was picked with a sterile needle, and the slant surface of one test tube was slightly streaked. The incubation was at 37^0C for 48 hr. Citrate positive result was interpreted by a colour change from green to deep blue, and there was no colour change in the negative result [12].

2.4.4 Indole test

This test determines the enzymatic removal of the amino group from tryptophan, A test tube containing 4 ml of tryptophan broth was taken and incubated aseptically by taking the growth from 18hr to 24hr culture, the tube was Incubated the tube at 37^0 C for 24 to 28hr. After incubation, 0.5ml of Kovac's reagent was added and shaken gently. A red ring formation at the surface of the tube indicated a positive test while yellow colour formation at the surface layer indicated a negative result [15].

2.4.5 Urease test

This test detects the enzymatic degradation of urea to carbon dioxide and ammonia. The media was autoclaved at 121^0C for 20 mins. The autoclaved media was poured into test tubes and kept in a rack in a slant condition after cooling. Bacterial colony was then inoculated using a sterilized wire loop by streaking on the surface of the test tubes, and incubated for 12 hr positive result was indicated by pink colouration [16].

2.4.6 Statistical analysis

The data obtained from laboratory analysis were analyzed using the Statistical Package for Social Sciences (SPSS), Statistics version 22 (IBM SPSS Inc. Chicago Illinois, USA). Mean and standard deviation are obtained using descriptive statistics.

3. RESULTS

Table 1. Bacteria count (cfu/ml) of *Telfairia occidentalis* and *Ocimum gratissimum*

Distribution (supply chain)	Types of Bags	(*Telfairia occidentalis*) Ugu	(*Ocimum gratissimum*) Nchuanwu
Farmland	Hessian Bag	1.93 ± 0.08^{a}	1.16 ± 0.08^{a}
	Non-Hessian bag	1.94 ± 0.06^{a}	1.16 ± 0.08^{a}
Distributor	Hessian Bag	2.04 ± 0.16^{a}	1.35 ± 0.08^{ac}
	Non-Hessian bag	2.43 ± 0.31^{ab}	1.40 ± 0.11^{ad}
Retailer	Hessian Bag	2.59 ± 0.86^{ac}	1.43 ± 0.10^{bcd}
	Non-Hessian bag	3.50 ± 0.99^{ae}	1.62 ± 0.02^{b}
Consumer	Hessian Bag	3.15 ± 1.08^{ab}	1.65 ± 0.07^{b}
	Non-Hessian bag	4.10 ± 1.11^{bcde}	1.79 ± 0.16^{b}

Values are means ± standard deviation ($\times10^4$) Alphabets with different Superscripts in the same column are statistically significant

Table 2. Coliform count (cfu/ml) of *Telfairia occidentalis* and *Ocimum gratissimum*

Distribution (supply chain)	Types of Bags	(*Telfairia occidentalis*) Ugu	(*Ocimum gratissimum*) Nchuanwu
Farmland	Hessian Bag	1.71 ± 0.01^{a}	2.1 ± 0.14^{a}
	Non-Hessian bag	1.73 ± 0.01^{a}	2.10 ± 0.41^{a}
Distributor	Hessian Bag	2.3 ± 0.71^{ac}	3.05 ± 0.49^{ab}
	Non-Hessian bag	2.75 ± 0.78^{agh}	3.40 ± 0.71^{b}
Retailer	Hessian Bag	2.7 ± 0.90^{ad}	3.75 ± 0.49^{b}
	Non-Hessian bag	3.45 ± 0.64^{bcdeg}	4.35 ± 0.64^{b}
Consumer	Hessian Bag	3.05 ± 0.90^{aef}	4.50 ± 0.28^{bd}
	Non-Hessian bag	4.20 ± 0.42^{bfh}	5.10 ± 0.28^{bc}

Values are means ± standard deviation ($\times10^4$) Alphabets with different Superscripts in the same column are statistically significant

Table 3. Fungal count (cfu/ml)of *Telfairia occidentalis* and *Ocimum gratissimum*

Distribution (supply chain)	Types of Bags	(*Telfairia occidentalis*) Ugu	(*Ocimum gratissimum*) Nchuanwu
Farmland	Hessian Bag	-	1.17 ± 0.03^{a}
	Non-Hessian bag	-	1.23 ± 0.01^{ab}
Distributor	Hessian Bag	-	1.36 ± 0.05^{b}
	Non-Hessian bag	-	1.39 ± 0.13^{be}
Retailer	Hessian Bag	1.28 ± 0.09^{a}	1.47 ± 0.06^{bc}
	Non-Hessian bag	1.39 ± 0.10^{a}	1.5 ± 0.06^{f}
Consumer	Hessian Bag	1.37 ± 0.04^{a}	1.55 ± 0.06^{cdfg}
	Non-Hessian bag	1.46 ± 0.06^{a}	1.59 ± 0.03^{g}

Values are means ± standard deviation ($\times10^4$) Alphabets with different Superscripts in the same column are statistically significant

3.1 Biochemical Characteristics of Microorganisms

Table 4. Bacterial isolates

Microorganisms	Gram reaction	Citrate	Catalase	Urease	Indole
Klebsiella oxytoca	Negative rods	Positive	Positive	Positive	Positive
Escherichia coli	Negative rods	Negative	Positive	Negative	Positive
Bacillus spp	Positive rods	Positive	Positive	Negative	Positive
Staphylococcus spp	Positive cocci	Positive	Positive	Positive	Positive
Aeromonas spp	Negative rods	Positive	Positive	Negative	Positive

Table 5. Distribution of bacterial isolates of the leafy vegetables in the supply chain from the farmland to the consumer

	HESSIAN BAG		NON- HESSIAN BAG	
Distribution (supply chain)	**(*Telfairia occidentalis*) Ugu**	**(*Ocimum gratissimum*) Nchuanwu**	**(*Telfairia occidentalis*) Ugu**	**(*Ocimum gratissimum*) Nchuanwu**
Farmland	*Staphylococcus spp*	*Staphylococcus spp*	*Staphylococcus spp*	*Staphylococcus spp*
Distributor	*Staphylococcus spp, Escherichia coli*	*Escherichia coli*	*Aeromonas spp, Staphylococcus spp*	*Bacillus spp Staphylococcus spp*
Retailer	*Bacillus spp, Pseudomonas spp*	*Bacillus spp, Pseudomonas spp*	*Bacillus, Staphylococcus spp*	*Staphylococcus spp*
Consumer	*Bacillus spp*	*Bacillus spp, Escherichia coli*	*Staphylococcus*	*Pseudomonas spp, Klebsiela oxytoca*

Table 6. Distribution of Fungi isolates of leafy vegetables in the supply chain from the farmland to the consumer

	HESSIAN BAG		NON- HESSIAN BAG	
Distribution (supply chain)	**(*Telfairia occidentalis*) Ugu**	**(*Ocimum gratissimum*) Nchuanwu**	**(*Telfairia occidentalis*) Ugu**	**(*Ocimum gratissimum*) Nchuanwu**
Farmland	-	*Aspergillus niger*	-	*Aspergillus niger*
Distributor	-	*Aspergillus niger*	-	*Aspergillus niger*
Retailer	*Aspergillus niger, penicillium spp*	*Aspergillus niger*	*Aspergillus niger, Mucor spp*	*Aspergillus niger*
Consumer	*Aspergillus niger, penicillium spp*	*Aspergillus niger*	*Aspergillus niger, Mucor spp*	*Aspergillus niger*

4. DISCUSSION

Bacteria, coliform and fungi isolates were found on the leafy vegetables in both the hessian bags and non-hessian bags. There was a continuous increase in the bacterial, coliform and fungal count in the supply chain from farmland to consumer during distribution as shown in Table 1, Table 2 and Table 3 respectively. Table 1 shows the respective means of *Telfairia occidentalis* distributed from farmland to consumer using hessian bag ranged from 1.93 × 10^4 to 3.15× 10^4 cfu/ml. Similarly, the distribution from farmland to consumer using non-hessian bags ranged from 1.94× 10^4 to 4.10× 10^4 cfu/ml. The respective means *Ocimum gratissimum* from farmland to consumer using hessian bags ranged from 1.16 × 10^4 to 1.65× 10^4 cfu/ml and non-hessian bags 1.16× 10^4 to 1.79× 10^4 cfu/ml. At (P> 0.05), there was no significant difference between the leafy vegetables (*Telfairia occidentalis* and *Ocimum gratissimum*) gottten from the farmland using hessian and non-hessian bags but at (P<0.05), there was significant difference between the leafy vegetable samples gotten from the distributor, retailer and consumer using hessian and non-hessian bag. The result showed that the bacteria count of *Telfairia occidentalis* and *Ocimum gratissimum* distributed using non-hessian bags are higher than those of hessian bags. This indicates that the hessian bag had a degree of anti-microbial activity probably as a result of its breathing ability. Along the supply chain, the bacteria count increased indicating that the various supply chain units are sources of contamination. Although bacteria colony multiplication over time could also be a factor. The result also show that *Telfairia occidentalis* has higher bacteria count than *Ocimum gratissimum* indicating that *Ocimum gratissimum* has more inherent anti-microbiological components than *Telfairia occidentalis* which can be harnessed.

Table 2 shows the coliform count of the leafy vegetables during distribution. Using the hessian bags, the coliform count in *Telfairia occidentalis* from farmland to consumer ranged from 1.71× 10^4 to 3.05× 10^4 cfu/ml and non-hessian bags 1.73× 10^4 to 4.20× 10^4 cfu/ml. The coliform count from farmland to consumer of *Ocimum gratissimum* using hessian bags ranged from 2.1× 10^4 to 4.5× 10^4 cfu/ml and non-hessian bags ranged from 2.1× 10^4 to 5.1 × 10^4 cfu/ml). At (P>0.05), there was no significant difference between the leafy vegetables (*Telfairia occidentalis* and *Ocimum gratissimum*) gotten from the farmland in the hessian and non-hessian bags and there was significant difference in the leafy vegetable samples gotten from the distributor and consumer in the hessian and non-hessian bags at (P<0.05) but there was no significant difference in *Ocimum gratissimum* leaves gotten from the distributor. Table 2 showed a similar result to Table 1 as there is a continuous increase in the microbial load from farmland to consumer in both hessian and non-hessian bags and the coliform count of *Telfairia occidentalis* and *Ocimum gratissimum* distributed using non-hessian bags are higher than those of hessian bags.

The fungal count of *Telfairia occidentalis* and *Ocimum gratissimum* are shown in Table 3. There were no fungi growth found in *Telfairia occidentalis* leaves collected from the farmland and distributor in hessian bags and non-hessian bags. 1.28× 10^4 cfu/ml and 1.37× 10^4 cfu/ml were found for the retailers and consumers using the hessian bags and 1.39× 10^4 cfu/ml and 1.46× 10^4cfu/ml in non-hessian bags. The fungi count in *Ocimum gratissimum* from farmland to consumer with hessian bags ranged from 1.17 × 10^4 to 1.55× 10^4 cfu/mland non-hessian bags from farmland to consumer ranged from 1.23× 10^4 to 1.59× 10^4 cfu/ml. At (P>0.05), there was no significant difference in retailer and consumer of *Telfairia occidentalis* leaves from hessian and non-hessian bags but at (P<0.05), there was significant difference in the *Ocimum gratissimum* leaves samples from the farmland, distributor, retailer and consumer in the hessian and non-hessian bags.

The biochemical characteristics of the bacteria isolates are shown in Table 4. The gram-positive cells were represented by the bacteria from the genera: *Bacillus spp,* while the gram-negative constituted members of *Klebiella oxytoca, Escherichia coli, Staphylococcus spp, Aeromonas spp,* this agrees with the previous reports by Guchi and Ashenafi [17]. The predominant microflora of fresh vegetables in the present study was generally *Bacillus spp* and *Staphylococcus spp. Klebiella oxytoca,* and *Staphylococcus spp* are citrate positive while *Escherichia coli, Aeromonas spp* are citrate negative, all the bacterial isolates are catalase positive, *Staphylococcus spp and Klebiella oxytoca,* urease positive and *Escherichia coli, Aeromonas, Bacillus spp* are urease negative. All the bacterial isolates are indole positive organisms.

Table 5 shows the occurrence of bacteria on the leafy vegetables during the distribution in the supply chain. The various bacteria isolates associated with the leafy vegetables usinghessian bags were *Staphylococcus spp, Escherichia coli, Bacillus and Pseudomonas* while non-hessian bagswere *Staphylococcus spp, Escherichia coli, Aeromonas, Bacillus, Klebsiela oxytoca and Pseudomonas.* The isolated organisms could be due to the microbial quality of irrigation water, because water contaminated with animal or human wastes products can introduce pathogens into vegetable products during preharvest and postharvest activities either directly or indirectly, therefore microbiological quality of irrigation water has a paramount importance to the safety of fresh and minimally processed vegetables [18]. Pathogenic microbes can adhere to the surfaces of the gloves worn by retail food employees and can serve as a source of cross contamination if not changed frequently [19].

The distribution of fungi isolates in the leafy vegetables using hessian bags and non-hessian bags is shown in Table 6. The organisms isolated along the supply chain in *Telfairia occidentalis* and *Ocimum gratissimum* were *Penicillium spp, Aspergillus niger,* for hessian bags and *Aspergillus niger, Fusarium spp,* and *Mucor spp* for non-hessian bags. There was no fungi growth from the farmland and distributor in both the hessian bag and non-hessian bag of *Telfairia occidentalis*. The emergence of fungal isolates on the leafy vegetables in the supply chain suggests possible contamination by spores in the air, since their spores are numerously available in air [20].

5. CONCLUSION

The result showed a progressive increase in bacteria and fungi count from the samples of farmland to the consumer indicating there are contaminations along the supply chain in accordance with [21]. Vegetables can become contaminated with human pathogens at multiple points along farm to table supply chain.

This study shows hessian bags does not have inherent anti-microbiological properties to reduce the microbial load in leafy vegetables during the distribution. Though hessian bags allow flow of air in and out of the bag thereby aerating its contents. This promotes escape of most free water thus preventing or minimizing rotting due to trapped moisture. This trapped moisture also encourages microbial activities.

We recommend that the farm workers, distributors, market retailers should maintain high levels of personal and environmental hygiene when handling the vegetables along the supply chain. We propose that designers should look at designing hessian bags with bactericidal and fungicidal properties. Studies on the biochemical properties and identification of the species of *Staphylococcus spp* present in the leafy vegetables should be done to ascertian the efficacy of the hessian bag.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oselebe HO, Nnamani CV, Okporie EO. Ethno botanical survey of underutilized crops and spices of some local communities in Nigeria: Potentials for improved nutrition, food security and poverty reduction. Iosr J Pharm. 2013;3:2250-3013.
2. Prior RL, Cao G. “Antioxidant photochemical in fruit and vegetables, diet and health implications.” Hort Science. 2000;35(4):588-59.
3. Murcia MA, Lopez Ayerra B, Martinez-Tome M, Vera AM, Garcia Carmona F. Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. J. Sci. Food Agric. 2000;80:1882-1886.
4. Barret DM. “Maximizing the nutritional value of fruits and vegetables.” Food Technology. 2007;61(4):40-45.
5. Masarirambi MT, Mavuso V1, Songwe VD, Nkambule TP, Mhazo N. Indigenous post-harvest handling and processing of

traditional vegetables in Swaziland: A review. African Journal of Agricultural Research. 2010;24(5):3333-3341.
6. Simpson JR, Weiner ESC. The oxford english dictionary. Oxford: Clarendon Press; 1989. ISBN 0-19-861186-2.
7. Djuikwo VN, Ejor RA, Gouado I, Mbofung CM, Tanumihardjo SA. Determination of major carotenoids in processed tropical leafy vegetables indigenous to Africa. Food and Nutrition Sciences. 2011;2:793-802.
8. Abetekassa G, Peterson HC. Market access for local food through the conventional food supply chain. Int food agricbusiness manage rev. 2011;4(1):41-60.
9. Mohanty AK, Khan MA, Henrichsen G. Influence of chemical surface modification on the properties of biodegradable Jute fabrics-polyesters amide composites. Composites part A. 2000;31:143-150.
10. Fawole MO, Oso BA. Laboratory manual of microbiology, spectrum books limited, Ibadan, Nigeria; 2001.
11. Cowan SST, Steel KKJ, Barrow GI, Feltham RKA. Cowan and steels manual for identification of the medical bacteria. 3rd Edn. Cambridge university press, Cambridge, UK. 1993;331. ISBN-13:9780521326117.
12. Agwung fobellah D, Kemajou ST. Laboratory microbiology and activities manual. Ark of wisdom publishers, Aba. 2007;51-52.
13. Cheesbrough M. District laboratory practice in tropical countries. Part 2. Low price Edition. Cambridge university press, Cambridge UK. 2006;180.
14. Mac Faddin JF. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams and Wilkins, Philadelphia, P.A; 2000.
15. Boor KJ. Pathogenic microorganisms associated with fresh produce. Food protection. 2005;25:767-770.
16. Boor KJ. Basic food microbiology. Diary food. Environmental sanitation. 1997;17(11):714-717.
17. Guchi B, Ashenafi M. "Microbial load, prevalence and antibiograms of *salmonella* and *shigella* in lettuce and green peppers." Ethiopian Journal of Health Sciences. 2010;20(1):43–47.
18. Solomon EB, Potenski CJ, Mattews KR. "Effect of irrigation method on transmission to method on transmission to and persistence of *Escherichia coli* on lettuce." Journal of Food Protection. 2002;65(4):673–676.
19. Lues JFR, Van Tonder I. "The occurrence of indicator bacteria on hands and aprons of food handlers in the delicatessen sections of a retail group." Food Control. 2007;18(4):326 –332.
20. Aurotupin DJ, Akinyosoye FA, Oladele OO. Microbial quality of selected commercially packaged chocolately beverages in Akure – Nigeria. Journal of Nigerian Society of Experimental Biology. 2003;3:34–41.
21. Mattew KR. Sources of enteric pathogen contamination of fruits and vegetables: Future directions of research. Stewart Postharvest.2003;9:1-5.

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