Direct RESEARCH JOURNALS
Vol.6 (1), pp. 13-18, January 2018
ISSN 2354-4147
DOI: https://doi.org/10.26765/DRJAFS.2017.3571
Article Number: DRJA10353571

Direct Research Journal of Agriculture and Food Science
http://directresearchpublisher.org/aboutjournal/drjafs

Research Paper

Microbial status of dried pepper (*capsicum spp.*), tomato (*Lycopersicum esculentus*), and roselle (*Hibiscus sabdariffa*) marketed in Minna, Niger State, Nigeria

Tsado[1*], E. K., Ekpa[2], D., Salwudeen[1], M. T., Adesina[1], O. A., Yusuf[1], S. T. and Izuegbu[1], L. N.

[1]Department of Crop Production, Federal University of Technology Minna, Niger State, Nigeria.
[2]Department of Crop Production and Protection, Federal University Dutsin-ma, Katsina State, Nigeria.
Corresponding author E-mail: ektsado@yahoo.co.uk.

Received 30 October 2017; Accepted 5 December, 2017

This study was carried out to evaluate the microbial load of dried pepper *(Capsicum* spp.*),* tomatoes *(Lycopersicum esculentus),* and roselle *(Hibiscus Sabdariffa)* sold at Minna, Niger state, Nigeria. An investigation was carried out for the isolation and identification of micro-organisms found in the selected dried vegetables. Total bacteria count in all the tested samples ranged from 2.0 x 10^4 to 7.0 x 10^4 cfu/g. Dried pepper showed the lowest amount of bacteria isolates/microbial load *(Proteus* spp. 7.0 x 10^4 and *Kleb spp.* 3.0 x 10^4) while the dry tomato showed the highest bacteria isolates *(E.coli* 3.0 x 10^4, *Staphylococus* 4.5 x 10^4 and *Bacillus subtilis* 3.5 x 10^4). Also in all the tested samples *Asperigillus fumigates* appeared as the most isolated fungi. It was clearly seen that all the tested samples were contaminated with different food borne pathogenic bacteria and fungi. Responsible authorities are advised to enlighten the public on how to maintain proper hygiene to avoid contaminations of these vegetables.

Key words: Micro-organisms, dried vegetables, food-borne pathogens

INTRODUCTION

Vegetables are imperative wellspring of dietary fibre, vitamins and minerals. They are comprised of powerful components of eating methodologies that can be utilized to improve our everyday exercises yet they are not promptly accessible lasting through the year because of regular variety (Ukegbu and Okereke, 2013). Juice from eating vegetables supplies essential advantages to the all-inclusive community when expended (Bhat *et al.*, 2011). At the point when nourishment that contains a lot of vegetables is consumed, it limits the danger of having some constant ailments, all around adjusted eating methodologies rich in vegetables ought to be set aside the vast majority of the opportunity to maintain a strategic distance from vitamin inadequacies of human body (Deanna and Jeffrey, 2007). Sufficiently devouring vegetables additionally help to control circulatory strain, restrain the danger of heart sicknesses, decrease blood cholesterol levels and dodge development of tumor in the body (Wiseman, 2008). Nigeria has issue of post-reap treatment of vegetables on account of high dampness content. Amid is the rare period i.e. at the point when the vegetables are out of seasons, the items ends up noticeably inaccessible along these lines constraining their use (Yacuik, 2001).

Creation of vegetables has been looked with a considerable measure of difficulties particularly as far as capacity, safeguarding, miniaturized scale life forms assault and so forth. A bigger amount of reaped natural products is however sundried bringing about low quality item with variable dampness content and microbial load that influences human utilization. Research has demonstrated that people that eat a great deal of vegetables when contrasted with the individuals who do exclude vegetables in their eating methodologies have a lower danger of creating stroke at the long run most particularly in provincial ranges, vegetables are vital wellspring of supplements since they contribute considerably to the protein, minerals, vitamins, fiber and supplement prerequisites which are more often than not in restricted supply in their eating regimens (Mosha and Gaga, 1999). Microbial tainting has been appeared to diminish a few supplements in vegetables particularly the dried one and this influences the nourishing estimation of the vegetables (Kordylas, 2002). As indicated by the world wellbeing association, the correct situation of sustenance borne sicknesses caused by polluted nourishment is 350times higher than the detailed cases (Hetzel *et al*., 2004).

Postharvest misfortunes of vegetables are hard to anticipate, the real operators delivering crumbling are those ascribed to physiological harm and blend of a few microorganisms (Flores, 2000). Because of the high rate of the interest for vegetables, it is extremely important to grow better technique for keeping item quality at a decent standard. Vegetable creation is looked with the issue of conservation and perishability which influences the time span of usability of the vegetables, (Nguyen *et al*., 2004). Likewise, microbial load introduce in vegetables additionally result to pollution in this way making hurt the customers. So many researchers have worked on tomato (*Lycopersicum esculentus*), pepper (*Capsicum spp*.) and Roselle (*Hibiscus sabdariffa*), but there appears to be a limited information on the microbial status of dried tomato (*Lycopersicum esculentus*), pepper (*Capsicum* spp.) and Roselle (*Hibiscus sabdariffa*), the level of contaminants present in these vegetables and how it has affected humans in the process of consumption especially as it affects this study area. The objectives of the study are to determine the level of microbial contamination on dried tomato (*Lycopersicum esculentus*), pepper (*Capsicum* spp.) and roselle (*Hibiscus sabdariffa*) marketed in Minna, Niger State and to isolate and identify the major types of bacterial found on sundried vegetables.

MATERIALS AND METHODS

Study location

The research work was carried out at the Microbiology Laboratory, Federal University of Technology, Bosso campus, Minna, Niger State of Nigeria.

Sample collection

Sun dried samples of three different vegetables; pepper (*Capsicum* spp.), Roselle (*Hibiscus sabdariffa*) and tomato (*Lycopersicum esculentus*) were collected from sellers of the dried vegetables at Kure market, Minna Niger State. The samples were labelled properly, packaged in samples collection bags and taken to the laboratory for microbial analysis.

Media composition and preparation

The used media were nutrient agar (NA) and Sabourad Dextrose agar (SDA) respectively (Table 1).

Preparation

Seven g of NA and 15.5 g of SDA were weighed using weighing balance (Top loading into 250 ml conical flasks each to make 250 ml of the media. Two hundred and fifty ml. measuring cylinder was used to measure distilled water which then dispensed into the Agar. It's then allowed to stand for 15 min to dissolve before autoclaving. Both media and peptone water which was used as a diluents were autoclaved at 121°C for 15 min after which it was allowed to cool to 0°C before it was brought out of the autoclave.

Serial dilution method/procedure

Nine ml of distilled water were Injected into the test tubes using 10 ml syringe and needle and then autoclaved. After autoclaving, it was allowed to cool and properly labelled with sample codes and dilution factor. One g of the sample was weighed and dispensed into the 1^{st} tube containing 9 ml of distilled water making it 10^{-1} then the sterilize syringe and needle was used to pipette 1ml from the 1^{st} tube to the 2^{nd} making it 10^{-2} up to 10^{-6} where some were applied to SDA for fungi and NA for bacteria. The bench is then disinfected using dettol and plates were labelled with the sample codes, media name, and dilution factor to b plate out for incubation.

Plating out

Another sterile syringe and needle was used to pipette 1 ml from 10^{-5} and 10^{-6} of each samples into Petri dishes according to their label for both NA and SDA respectively, then the media which was allowed to cool to 45°C was then aseptically poured into plates and placed well on the

Table. 1 Media composition

Materials used	Media composition
Composition of nutrient agar in grams/liters	
Beef extract	3.0
Peptone	5.0
Agar	12.0
Sodium chloride	8.0
Composition of the SDA in grams/liters	
Balanced peotone	10.0
Dextrose	40.0
Agar no 2	12.0

table for proper mixing of sample and media (the method is referred to as pour plate method),it was then left on the table for some time for it to jell or solidify before putting NA plates into incubator for 24 h at 37°C.After that, it was viewed and colonies were counted and taken. Similarly, SDA plates were incubated at 25°C for 72 h, after which the growth were viewed and counted.

Total viable count

From the plates of serial dilution technique, plates out of (10^{-5} and 10^{-6}) which were incubated at 37°C for 24 h and 72 h at 25°C for bacteria and fungi respectively, serial dilutions which yield germs more than 300 colony were chosen for count. The colony counting matching was used to count it with the help of its light source which illuminates and magnify the colony trough magnifying lenses, the plates were placed in inverted position on the counter and light was on, each colony was counted by marking them with pinching pen, for easier and convenient count. Plates was divided into 4 chambers, after counting the number of colonies in a chamber, it was then multiplied by 4 to give the total number of microorganisms present in that plate. From the plate count data, the concentration of bacteria/fungi in the original sample was calculated using the formula below:
Total no of colony in a plate X the reciprocal of dilution factor

e.g. If the colonies count in a plate is 150 colonies

$$= 150 \times \frac{1}{10^{-5}}$$

$$=150\times \frac{1}{1} \times \frac{1}{\frac{10^{-5}}{1}}$$

$$= 150\times10^{5}$$

$$=1.50\times 10^{7}\text{c.f.u/grams}$$

The result is expressed in cfu/g or cfu/ml in case of solid and liquid respectively.

c.f.u; means colony forming units.

This means 1 g or 1 ml of the sample contains 1.50×10^{7} cfu/g. The higher the dilution, the fewer the no of colony was observed and vise- versa.

Bacteria identification

The bacterial spread was recoloured with 0.5% precious stone violet for 2 min. It was then washed with water, depleted off and recoloured with weaken iodine for 2 min. The precious stone violet and iodine shape a purple/dark complex inside the bacterial cell. A trickle of supreme liquor was painstakingly dropped unto the spread and permitted to keep running off. This was rehashed three times and washed off with water (the liquor breaks down the lipid encompassing the gram negative cells and permit the gem violet and iodine complex to wash off). It was counter stain with 1% safranin for 2 min and after that washed and slide dried. The slide was seen under a magnifying instrument and cells which were recoloured purple or dark were distinguished as gram positive bacterial cells while those recoloured light pink were recognized as gram negative bacterial cells.

Statistical analysis

All information gathered from the test were subjected to measurable investigation of fluctuation utilizing Minitab discharge 18. Contrasts among treatment implies were isolated utilizing LSD test at 5% (p=0.05).

RESULTS

Table 2 shows bacteria species isolates from the different died vegetable samples with the most isolates been Bacillus subtlis. Bacillus is a gram positive bacterium with rod-shape. This microorganism was found in the dry tomato and the dry roselle, with the dry roselle having the

Table 2. Bacteria isolates/cfu/ml.

Sample	Isolates name	TVC	TCC	TSS
Tomato	*E. coli*	3.0×10^4	2.0×10^4	-
	Staph aureus	4.5×10^4	-	-
	Bacillus spp.	2.5×10^5	-	-
	Lacto bacillus	4.9×10^5	-	-
	Bacillus subtlis	3.5×10^4	-	-
Roselle	*Staph aureus*	5.5×10^4	-	-
	Pseudomonas aenigeria	3.0×10^4	-	-
Pepper	*Bacillus subtlis*	4.2×10^5	-	-
	Proteus spp.	7.0×10^4	-	-
	Kleb spp.	3.0×10^4	-	-

KEY: TVC Total viable Count; TCC: Total coliform count; TSS: Total salmonella count; - No growth of TSS from samples.

Table 3. Fungi count cfu/ml.

Sample	Isolate	TVC
Tomato	*Aspergillus niger*	5.0×10^2
	Aspergillus fumigates	7.0×10^2
	Aspergillus flavus	3.0×10^2
Roselle	*Trichophyton tonsurans*	2.0×10^2
	Trichophyton soudanense	3.0×10^2
	Aspergillus niger	1.0×10^2
Pepper	*Aspergillus fumigates*	7.0×10^2
	Aspergillus fumigates	2.0×10^2
	Aspergillus niger	1.0×10^2
	Mucor spp.	6.0×10^2

Table 4. Fungi count cfu/ml.

Sample	Isolate	TVC
Tomato	*Aspergillus niger*	5.0×10^2
	Aspergillus fumigates	7.0×10^2
	Aspergillus flavus	3.0×10^2
Roselle	*Trichophyton tonsurans*	2.0×10^2
	Trichophyton soudanense	3.0×10^2
	Aspergillus niger	1.0×10^2
Pepper	*Aspergillus fumigates*	7.0×10^2
	Aspergillus fumigates	2.0×10^2
	Aspergillus niger	1.0×10^2
	Mucor spp.	6.0×10^2

highest amount of this bacterium. The outcome displayed in (Tables 3 and 4) show organisms species disengages from the dried vegetable example with Aspergillums treats been the most confined microorganism. *Aspergillus spp.* are known to deliver aflotaxins to fluctuating degrees when they are developed on media. This microbial subsidizing is like some prior reports (Makun *et al*., 2012). The vacillation among the organisms contaminants with that found in other existing literary works came up because of contrasts in nation of source, related outer elements, test change, the technique for handling storerooms in a specific area (Garcia *et al*., 2001).

DISCUSSION

In this present study, all the dried vegetables were found infected. Microbial load associated with the different samples are presented in (Table 2).

Total bacterial count (TBC)

In the present research, microbial quality of 3 different dried vegetables which include pepper (*Capsicum* spp.), tomato (*Lycopersicum esculatum)*, and roselle (*Hibiscus sabdariffa*) were determined. Total bacteria count in all

the tested samples (Table 2), ranged from 2.0 x 10 to 7.0 x 10^4 cfu/g – in all the samples, the dry pepper showed the lowest amount of bacteria isolates/microbial load (*Proteus* spp. 7.0 x 10^4 and *Kleb* spp. 3.0 x 10^4) while the dry tomato showed the highest bacteria isolates (*E.coli* 3.0 x 10^4, *Staphillococus* 4.5 x 10^4, *Bacllus* spp. 2.5 x 10^5, *Lacto bacillus* 4.9 x 10^5 and Bacillus sub tills 3.5 x 10^4). This outcome shows that dried vegetables predominantly tomato is polluted and the sullying may be because of dishonorable treatment of the vegetables, the drying technique received, the condition that was utilized as a part of the reason for drying the vegetables and individual cleanliness of the merchants (Tambekar *et al.,* 2009). The after effect of this investigation likewise elucidate that pepper and roselle were observed to be focus manted with less number of microbes, this could be because of the basic and acidic nature separately.

Total viable count (TYC)

The total viable count of fungi species found in the tested samples ranged from 1.0 10^2 to 7.0 x 10^2 cfu/g. The dry Roselle showed the lowest amount of fungi species which ranged from 1.0 x 10^2 to 3.0 x 10^2 of *Aspergillus niger* and *Trichophyton tonsurans* respectively. While tomato (*Lycopersicon esculentus*) showed the highest amount of fungi species which ranged from 3.0 x 10^2 to 7.0 x 10^2 for *Aspergillus niger* and *Aspergillus fumigates* respectively. Predominantly, the most isolated fungi species are the *Aspergillus spp.* which includes *Aspergillus niger, Aspergillus fumigates* and *Aspergillus flavus*. This sullying could be because of characteristic contamination with of the climatic state of Minna. The investigation territory is caution scope yearly temperature of 31.7°C and damp scope yearly stickiness of 51.6% most circumstances of the year, which are positive conditions for the development of growths (Ominski *et al*., 1994). Minna advertise - where development of individuals and vehicles are in kept spaces - may result to increment in tidy and microbial extra for movement (Fandohan *et al*., 2006) that will expand parasites advancement. Likewise the defilement could likewise capacity of these vegetables (Chourasia, 1995). The level of organisms invasion can be exacerbated by creepy crawly harm, stretch condition at both pre-reap, collect and post-gather phases of vegetable creation (Lamboni and Hell, 2009).

Identification of micro-organisms

For identification, different bacterial and isolates were selected from different media on the basis of agar colongi morphologiga and biochemical characteristics. A total of 14 different organisms were identified (Both bacteria and fungi spp) from the samples including *Escherichia coli*, *Staphylococcus spp.*, *Bacillus* spp., *Klebsiella*, *Proteus*, *Lacto bacillus*, *Pseudomon* as *aerugena*, *Aspergillus spp*, *Trchophyton tonsurans* and *Mucor* spp.*, Bacillus* spp. and *Aspergillus* spp. were the most frequently isolated being present in all the examined vegetables.

Vanderzant and Splittstoesser (1992) showed that *Pseudomonas* spp. and *Bacillus* spp. were the most widely recognized vegetable deterioration microbes. The event of *Staphylococcus* spp., *E. coli* and *Bacillus* spp. are ordinarily connected with parsanitan, hones (Oranusi *et al*., 2006). Be that as it may, encompassing temperature may build the microscopic organisms checks of vegetables (Bryan, 1977). Vanderzant and Kaur, (2001) demonstrated the nearness of salmonella, *Staphylococcus* spp. and *Pseudomonas aeruginosa* in vegetables and organic products.

Conclusion

The result of this study clearly indicated that the dried pepper (*Capsicum* spp.), tomato (*Copersicum esculentus*) and Roselle (*Hibiscus sabdariffa*) are contaminated with different food borne pathogenic bacteria and fungi which include *Proteus*, *Klebsilla*, *Escherichia coli*, *Staphylococcus* spp., *Bacillus* spp., *Aspergillus* spp., *Trichophyton tonsurans* spp. and *Mucor* spp. the presence of possible pathogenic species on food products carries a potential public hazard to the consumers. The contamination might be due to improper washing of the vegetables, poor hygienic conditions, prolonged preservation at ambient environment with swarming flies, locating shops in open air and lack of basic safety issues by vendors etc.

Recommendations

It is suggested that dependable experts should grasp measures and polices through down to earth instruction and re-preparing programs for sustenance business operations at all means of nourishment generation stages (from creation to utilization, particularly locally situated makers). Likewise, once these measures are set up, they require checking and implementation through obviously paper nourishment laws and controls. The discoveries from this investigation can be profitable for other hazard appraisal of the effect on human wellbeing of expending nourishments particularly dried vegetables.

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