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Isolation of Bacteria from Onions with Brown Rot Symptoms in Enugu State, Nigeria

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A B S T R A C T

The study was conducted to investigate bacteria associated with the spoilage of onion bulbs vended in parts of Enugu State, Nigeria. A total of 20 samples were collected from Enugu main market. Samples were cultured on nutrient agar and isolates identified using standard methods based on their microbial and biochemical characteristics. Nutritional and proximate analysis was carried out using standard methods of AOAC, (2010). The bacteria species isolated were *Bacillus subtilis*, *Shigella spp*, and *Escherichia coli. Escherichia coli* had the highest occurrence at (15)75% while *Shigella spp* occurred lowest at (5)25%. The proximate analysis showed high amount of moisture content at 73.99 ± 0.14^{aa} while ash content had the least at 0.26±0.30^{aa}. Mineral Composition showed high amount of potassium at 67.90mg/kg while lead and copper occurred least at 0.01mg/kg and 0.18 mg/kg respectively. The pathogenicity test on the onions showed that *Bacillus subtilis* and *Escherichia coli* were the causes of spoilage on onions. There is need to develop new acceptable pre-harvest and post-harvest disinfection methods.

Introduction

The onion (*Allium cepa* L., from Latin *cepa* "onion"), also known as the bulb onion or common onion, is a vegetable and is the most widely cultivated species of the genus Allium. Its close relatives include the garlic, shallot, leek, chive, and Chinese onion. This genus also contains several other species variously referred to as onions and cultivated for food, such as the Japanese bunching onion (*Allium fistulosum*), the tree onion (*A. proliferum*), and the Canada onion (*Allium canadense*). The name "wild onion" is applied to a number of *Allium* species, but *A. cepa* is exclusively known from cultivation. Its ancestral wild original form is not known, although escapes from cultivation have become established in some regions. The onion is most frequently a biennial or a perennial plant, but is usually treated as an annual and harvested in its first growing season (Lukes, 1986).

The onion plant has a fan of hollow, bluish-green leaves and its bulb at the base of the plant begins to swell when a certain day-

length is reached. The bulbs are composed of shortened, compressed, underground stems surrounded by fleshy modified scale (leaves) that envelop a central bud at the tip of the stem. In the autumn (or in spring, in the case of overwintering onions), the foliage dies down and the outer layers of the bulb become dry and brittle. The crop is harvested and dried and the onions are ready for use or storage. Onions are cultivated and used around the world. As a food item, they are usually served cooked, as a vegetable or part of a prepared savoury dish, but can also be eaten raw or used to make pickles or chutneys. They are pungent when chopped and contain certain chemical substances which irritate the eyes.Due to its significant medicinal property, nutritional worth and energy value, onions (*Allium cepa* L.) impart numerous health benefits to users. The crop is prone to attack by a number of pests and diseases, particularly the onion fly, the onion eelworm, and various fungi which rot.

Statement of problem

Onions are often consumed raw and are part of our daily diet, there are often signs of spoilage on them and are used irrespective of this; hence the burden of this work.

The main aim of this study was designed to isolate bacteria associated with partly spoilt onions, determine the pathogenic isolates capable of causing spoilage and determine the proximate and mineral composition of fresh onions in parts of Enugu State Nigeria.

Materials and Methods

Collection of samples

A total of 20 bulbs showing brown rots were purchased from local retailers in Ogbete main market in Enugu metropolis. The onion samples were collected in clean polyethylene bags and taken to microbiology laboratory, ESUT.

Isolation of bacteria from onions

Nutrient, MacConkey and Manitol salt agar (all from Oxoid, England) were prepared according to manufacturer's instruction and sterilized by autoclaving at 121^0C for 15 min. The onions bulbs showing signs of brown rots were first sterilized with 95% alcohol and washed off with sterile water to reduce the effect of the alcohol. Sterile surgical blend was used to cut off the soft rot and were blended using the sterile surgical blender. A homogenate of each sample was made by blending 10g in 25ml of sterile water. Serial dilutions of 10^{-1}-10^{-10} were made in sterile test tubes by several transfers of 1ml of previously diluted samples from 1st dilution tube to 9ml of sterile water in subsequent tube. The tubes were labeled appropriately as diluted. 0.1ml of 10^5 dilution was inoculated on the different agar using pour plate method. They were allowed to solidify, inverted and incubated at 37^0C for 24 h for colony formation. Each colony was isolated in a pure form by sub-culturing for further studies and identification. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Further microbial identification was based on the methods of Jolt *et al.,* (1994).

Pathogenicity test

Healthy onions were surface sterilized with ethanol for 1 min and washed in five changes of distilled water. Each onion was weighed and readings were recorded. 5 ml cork borer was punched to a depth of 4mm into the healthy onion bulb and the bored tissues were removed. Sterility test was conducted on the extracted onion tissues to determine the presence of microorganism prior to inoculation with isolates. This was done by

culturing on nutrient agar at 28°C for 24h. Pure cultures of each isolated bacteria were taken from the plate with the aid of wire loop and were placed back into the bored hole on each bulb. The wounds were sealed with prepared candle wax according to the method of Fawole and Oso (1998). Control experiment was set up in the same manner except that sterile agar disc was used instead of inoculums. The inoculated onions were placed at room temperature under sterile condition. After 96h, the onion with inoculum introduction was observed for signs of spoilage. The spoilt parts were scooped off, and then reweighed. Three (3) replicates were carried out for each inoculum. To determine the severity of rots caused by these organisms, the formula illustrated by Chukwu *et al.*, (2010), was used based on the weight values recorded from pathogenicity test procedure.

% pathogenicity=(Wx100%)/Wy

Where W = number of infected onion bulbs
Wy = total number of sample.

Proximate analysis

The method of AOAC (2016), AOAC (2007) and ISO 17025:2005 were used and the analysis involved the determination of the % constituents of the parameters; Moisture Content: 1 gram of the sample was weighed into a crucible and dried in an oven for 1 hour at 105˚c.% moisture content loss = W1 – W2/ WT ×100/1. Ash content: 1 gram of the fruit sample was weighed and ashed using a muffle furnace at 500˚c. Ash content W2 – W1% Ash content W2 – W1/WT ×100/1.

Crude Fiber Content: 2 grams of the fruit sample undergone acid treatment and base treatment and finally taken for ashing. Weight of fiber = weight of residue – weight of ash, % crude fiber = fiber weight/sample weight × 100/weight. Protein Content: 0.5gram of the sample was weighed into kjethahyl flask for digestion, distillation and finally titration. TV × 0.0014 × 6.25 × DF × 100/Weight of sample. Fat Content (Lipid – Fat & Oil): 5grams of the sample was weighed and the extraction of fat was done using soxhlet extraction method. Weight of oil W2-W1% lipids = W2 – W1/Weight of sample × 100/1

Mineral analysis

The elemental analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA 1995 (America Public Health Association) and the American Public Health Association (1998) 2111B, Direct Air – Acetylene Flame method.

The digestion was carried out according to AOAC International (2007), Official methods of analysis 18th edition (2005) and method 960.52 (Micro-kjeldahl method) an (method 992.23 (Generic Combustion Method).

Statistical analysis

The SPSS version 21.00 was used for the data analysis, statistically significant different groups was calculated with the One-way Analysis of variance (ANOVA). The Duncan test was used for multiple comparisons, and level of statistical significance was set at $p \leq 0.05$.

Results and Discussion

Out of 20 slightly spoilt onion samples, *Escherichia coli* were highest in occurrence at 15(75%) while *Shigella spp* occurred least at 5(25%) (Table 1).

Both *Escherichia coli* and *Shigella spp* were Gram negative rods while *Bacillus subtilis* were Gram positive short rods. The organisms differed in their biochemical characteristics (Table 2).

Table.1 Occurrence of bacteria present in onion with brown rot

Number of sample collected	Organism isolated	Number of positive (%)	Number of negative (%)
20	*Escherichia coli*	*15 (75%)*	*5(25%)*
20	*Bacillus subtilis*	*10 (50%)*	*10(50%)*
20	*Shigella spp*	*5 (25%)*	*15(75%)*

Table.2 Microscopic and biochemical characteristics of bacteria isolates

(1) Cultural Characteristics			
Colour	Creamy	White	Creamy
Shape	Circular	Large	Irregular
(2)Morphological characteristics			
Cell type	Rod	Rod	Rod
Cell arrangement	Single	Single	Single
(3)Gram stain	_	+	_
(4)Motility test	+	_	+
(5)Biochemical tests			
Catalase	_	+	+
Indole	_	_	+
Coagulase	_	+	_
Methyl red	_	_	+
Citrate	-	_	_
VP (Voges Proskauer)	-	+	+
Simmons citrate agar	-	+	-
(6)Sugar fermentation test			
Glucose	Acid and gas	Acid and gas	Acid and gas
Lactose	Acid and gas	Acid and gas	_
Probable microscopic identity	*Shigella spp.*	*Bacillus spp.*	*Escherichia coli*

Table.3 Pathogenicity test of bacterial isolates on whole onions

Causative organism	Total Number of Sample	Number of infected samples
Escherichia coli	20	17 (85%)
Bacillus subtilis	20	12 (60%)
Shigella spp	20	-

Table.4 Proximate analysis of fresh onion bulbs

Sample	Proximate Parameters	Composition %
Fresh Onions	Moisture	73.99 ± 0.14^{aa}
	Ash	0.26 ± 0.30^{bb}
	Protein	3.85 ± 0.03^{bb}
	Crude fat	6.43 ± 0.02^{bb}
	Fibre	3.46 ± 0.01^{bb}
	Carbohydrate	12.01 ± 0.06^{aa}
	Energy Value	6.43 ± 0.05^{bb}

Means with the same superscript were not significantly different at $p<0.05$. The means were separated using least significant difference (LSD)

Table.5 Mineral analysis of fresh onion bulb

Sample	Mineral (mg/kg)	Composition %
Fresh onions	Calcium	31.34 ± 1.22^{aa}
	Iron	2.10 ± 0.03^{bb}
	Zinc	0.85 ± 0.07^{bb}
	Copper	0.18 ± 0.01^{aa}
	Sodium	6.10 ± 0.07^{bb}
	Potassium	67.90 ± 0.19^{aa}
	Magnesium	1.60 ± 0.03^{bb}
	Lead	0.01 ± 0.00^{bb}

Means with the same superscript were not significantly different at $p<0.05$. The means were separated using least significant difference (LSD)

The pathogenicity test showed that *Escherichia coli* and *Bacillus subtilis* were capable of causing spoilage on onions at 17(85%) and 12 (60%) respectively. *Shigella spp* were not able to cause spoilage on onions (Table 4).

Proximate parameters showed varying results with moisture having the highest content at 73.99%.

Potassium was present in fresh onions and had very high content at 67.90±0.19.Other mineral compounds varied in their compositions (Table 4).

Growth of some microorganisms takes place between harvesting and processing or consumption of vegetables, (Frazier and Westhoff, 1987). From the study, out of the 20 samples studied, *Escherichia coli* occurred in15 (75%), *Bacillus subtilis* in 5 (25%) and *Shigella spp* in 10 (50%) (Table 1). This supports the work of Orpin (2017) who isolated *Staphylococcus spp, Bacillus spp, Pseudomonas spp,* and *Escherichia coli* from onions. It is also in line with the works of Shinkafi and Dauda, (2013) who also isolated *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus pneumonia* from onions. In the present study, the pathogenicity test of the bacterial isolates from whole onions showed that *Escherichia coli* and *Bacillus subtilis* initiated the spoilage of the onions. The prevalence of the pathogenicity in the present study showed 17 (85%) for *Escherichia coli* and 12 (60%) for *Bacillus subtilis* while *Shigella spp* were not found to be involved agents of spoilage on onions. This supports the work of Orpin *et al.,* (2017) who identified *Bacillus spp, Pseudomonas spp and Escherichia coli* as pathogenic causes of spoilage in onions.

The proximate compositions of *Allium cepa* L. showed a considerable quantity of carbohydrate (12.01%) (Table 5). The values obtained in the study is in accordance with the work of Shovon *et al.,* (2013) who obtained carbohydrate values at 14.146%. Carbohydrates are energy giving food and are also needed in numerous biochemical metabolisms not directly concerned with energy metabolism and may serve as substrates for the production of aromatic amino acids and phenolic compounds through the Shikimic acid pathway and this may confer high phenolic and antioxidant potentials (Ponnusamy *et al*., 2012). Thus the carbohydrate levels of the studied samples suggest its usefulness as alternative source of glucose. The level of protein in the test sample of *Allium cepa* L. in the present study was 3.85 %. The protein values were similar to those of Shovon *et al.,* (2013) who found protein at 2.62 % from onions samples. Protein intake can contribute to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance (replacement of wear and tear of tissues) of body. Crude fiber is increasingly being recognized as a useful tool for the control of oxidative processes in food products and as functional food ingredient. In the present study, the contraction of muscular walls of the digestive tract is stimulated by fiber, thus counteracting constipation (Ponnusamy and Vellaichamy, 2012).

The presence of crude fiber in the diet is necessary for digestion and for elimination of wastes and occurred at 3.46% in the present study. In addition, it decreases the absorption of cholesterol from the gut in addition to delaying the digestion and conversion of starch to simple sugars, an important factor in the management of diabetes. It may reduce cerium cholesterol levels too. Crude fiber also functions in the protection against cardiovascular disease, colorectal cancer and obesity (Ponnusamy and Vellaichamy, 2012). In the study, proximate composition also shows high moisture content of 73.99 ± 0.14^{aa} (Table 5). This is in line with the work of Shovon *et al.,* (2013) who found the proximate composition of moisture content high at 82.99%, while the ash content was significantly low at 0.26 ± 0.30^{aa} (Table 5). The moisture content enables the growth of the pathogenic microorganism which facilitates the spoilage in onions. There was statistical

difference at $p<0.05$ between carbohydrate, protein, moisture content and ash.

From the study, the mineral composition of whole onions showed that potassium occurred highest at 67.90mg while zinc, copper and lead occurred least at 0.8mg, 0.18mg and 0.01mg respectively. This is also in line with the work of Shovon *et al.*, (2013) who obtained high values of potassium at (140mg and 129mg respectively from two varieties of onions. There was statistical difference at $p<0.05$ between values of potassium, zinc, copper and lead.

The mineral composition found in the study supports the works of Ogbonna *et al.*, (2016) who found the mineral compositions of potassium (159 mg/kg), calcium (222 mg/kg), magnesium (211.3 mg/kg), iron (0.31 mg/kg), zinc (0.21 mg/kg) and sodium (3.2 mg/kg). It is also supports the work of Irene *et al.*, (2008) who found calcium (175.0 mg/100 g), potassium (1010 mg/100 g) and low amount of sodium (11.2 mg/100 g).

In conclusion, onions samples with brown rot were contaminated with *Escherichia coli*, *Bacillus subtilis and Shigella spp*. *Escherichia coli* and *Bacillus subtilis* were found to be pathogenic causes of spoilage on whole onions sold in Agbani, Enugu State, Nigeria. Onions were also found to be rich sources of minerals and have a high energy value.

There is need to develop new acceptable pre-harvest and post-harvest disinfection methods. This will help farmers and consumers take necessary precaution in preventing contamination of onions, thus reducing the risk of infection with toxins which are harmful to human.

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