

Bacteriological-profile of some vegetables sold in Lafia Metropolis, Nasarawa State, Nigeria

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Abstract

The bacteriological-profile of fresh spinach and cabbage sold in Lafia Modern Market were analyzed in the Microbiology laboratory of Nasarawa State Polytechnic, Lafia by homogenizing 1g of the sample in 10 ml of peptone water. An aliquot from a 10-fold serial dilution was inoculated using the pour plate method into different bacteriological medium at 37°C for 24 hours. Total number of colonies was counted and identified using standard procedures. Results indicated that spinach had the highest average bacteria count of 1.4 x10⁴CFU/g, 9.2x10³CFU/g and 6.1x10³CFU/g Nutrient agar, MacConkey agar and Salmonella-Shigella agar respectively, while cabbage had an average bacterial count of 1.0x10⁴CFU/g, 6.0x10³CFU/g and 4.1x10³ CFU/g on Nutrient agar, MacConkey agar and Salmonella-Shigella agar respectively. The genera of the bacteria isolates identified were *Streptococcus* spp (8%), *Bacillus subtilis* and *Pseudomonas aeruginosa* (15% each), *Staphylococcus aureus* (16%), *Escherichia coli* and *Salmonella* spp (23% each). These isolated bacteria are of public health importance due to their implication in food borne illnesses. It is recommended that hygiene-level of the entire vegetable processing value chain should be improved upon in order to prevent or reduce bacterial contamination.

1. Introduction

Vegetables are plants rich in essential bioactive nutrients like minerals, fiber and vitamins (Conner, *et al.*, 2017), they are consumed (raw or preheated) by ruminant animals and humans (de Evan, *et al.*, 2019). Most vegetables are usually green plants e.g. spinach, lettuce, and pumpkin, while others such as cabbage, onion, mushroom and radish, are non-green vegetable plants (Amao, 2018). Vegetables are very fragile in nature, therefore in order

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not to lose their unique nutrients, it is recommended that consumers eat them raw or slightly-heated, as too much heat resulting from over cooking, will destroy their nutrients (Chaturvedi, *et al.*, 2013; Feng, *et al.*, 2022).

The health benefits derived from consuming fresh vegetables containing high fiber and vitamins content make them more popular for the people who care about boosting their immunity using proper diet especially in the post-COVID pandemic era (Chowdhury, 2020).

Spinach (*Spinacia oleracea*) is an edible fiber-rich, low-calories, flowering vegetable plant belonging to the family *amaranthaceae*, native to central and western Asia (Britannica, 2022; Hedges and Lister, 2007). It grows fastest in well-drained soil rich in organic matter such as compost manure and with a pH of 6.5 to 7 (Warid, 2018). In order to grow spinach twice a year, it is planted about 4 to 6 weeks before the last frost in the spring, and again 6 to 8 weeks before the first frost in the fall and the plants are spaced 12 inches apart, this gives leaves room to reach full size (Ozlem and Sener, 2005). Spinach is best known for being an extremely rich source of phytochemicals (like lutein, phenolic compounds, zeaxanthin, and β -Carotene) and core nutrients (Hedges and Lister, 2007). The leaves are often used in making various vegetable delicacies, its main micronutrients is vitamins A (from β -Carotene), C and K, as well as folate and minerals such as calcium, iron and potassium. Other nutrients present in smaller quantities include vitamin E, some B vitamins -Thiamine (B₁), riboflavin (B₂) and the minerals magnesium, manganese and zinc (Athar *et al.*, 2004b; Hedges and Lister, 2007).

Cabbage (*Brassica oleracea* var. *capitata*) is an important vegetable known to mankind for over 4,000 years (Teshome, *et al.*, 2018). It is a member of the mustard or cruciferous family (*Brassicaceae*), which includes mustard, rape, turnip, wasabi (*Eutrema wasabi*), radish, watercress, many oriental vegetables, and a very important model plant *Arabidopsis thaliana* (Shrestha, 2019). In terms of life cycle, cabbage is a short lived perennial crop, usually biennial. Cabbage grows best on well-drained fertilized soils with constant availability of adequate moisture and under moderate temperature and pH in the range 6.0 - 6.5. It is essential not to grow cabbage on the same field year after year because of accumulation of various pathogens, to which crops is highly susceptible (Tsoho and Salau, 2012).

Cabbage contains calcium in the range of 22-150mg/100g. Its accumulated mineral source is at very high level of phosphorus, sulphur, chlorine, calcium, iron and potassium (Jahangir *et al.*, 2009). Cabbage comprises potentially useful amount of copper, zinc and a number of other important minerals and trace elements. Cabbage has a lot of health benefits

which includes prevention of oxidative stress, induction of detoxificative enzymes, and stimulation of immune system reduction of cancer cells and inhibits malignant transformation and carcinogenic mutation. It also plays an important role in the etipathology of many diseases such as vasospasm, atherosclerosis, cancer, heart attack, stroke and liver damage (Athar, *et al.*, 2004a).

Although the consumption of vegetable products has increased in recent years (Feng, *et al.*, 2022), these vegetables have also become vehicles for the transmission of some kinds of pathogens when eaten raw from unhygienic preparation causing food poisoning (Chaturvedi, *et al.*, 2013; Chowdhury, 2020). They are widely exposed to microbial contaminations through contact with water, soil, dust, and by handling at harvest or during post harvest processing. They therefore harbor both human and plant pathogens (Teshome, *et al.*, 2018). Pathogenic bacteria that have been detected in fresh vegetables (Spinach and cabbage) are coliform bacteria, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp (Tambekar and Mundhada, 2006).

This study therefore aims to identify and compare the bacteriological load of cabbage and spinach sold in Lafia modern market in Nasarawa State, North-Central Nigeria.

2. Materials and Methods

2.1. Sample Collection

Fresh spinach (*Spinacia oleracea*) and cabbage (*Brassica oleraceaver-capitata*) vegetables were purchased from modern market Lafia, Nasarawa State, North-Central Nigeria.

2.2. Sample Preparation and Dilution

1 grams (1g) of each sample was weighed using a weighing balance, centrifuged and homogenized in 10 ml of peptone water for 10mins. A 10-fold serial dilution was done using 1ml of the homogenate and 9 ml of sterile distilled water in five test tubes.

2.3. Enumeration of Bacterial Dilution

An aliquot was taken from each dilution and was inoculated into the following bacteriological medium (Nutrient agar, MacConkey agar and *Salmonella-Shigella* agar) using the pour plate method, and subsequently incubated at 37⁰C for 24hours. The total number of

colonies were counted and differentiated on the basis of their morphology and counts were obtained from different colonies (Oleghe *et al.*, 2022).

2.4. Preparation of Pure Culture

Pure cultures of representative's bacteria colonies were obtained by sub-culturing and streaking onto sterile freshly prepared nutrient agar. The plates were then incubated and maintained in agar slants at 37⁰C for 24 hours.

2.5 Characterization of Isolate and identification

Identification of isolates was confirmed by Gram staining, cultural, morphological and biochemical characterization using routine laboratory techniques according to Oleghe *et al.*, (2020). All analyses were performed in triplicate.

3. Results and Discussion

The results of the plate count indicated that spinach had the highest average bacteria count of 1.4 x10⁴CFU/g, 9.2x10³CFU/g and 6.1x10³CFU/g on Nutrient agar, MacConkey agar and *Salmonella-Shigella* agar respectively, while cabbage had an average bacterial count of 1.0x10⁴CFU/g, 6.0x10³CFU/g and 4.1x10³ CFU/g on Nutrient agar, MacConkey agar and *Salmonella-Shigella* agar respectively (Table 1; Figure 1).

Table 1: Average Total Count of Bacteria Isolate in the Samples on Some Vegetables Sold In Lafia in Colony forming unit per gram (CFU/g).

<i>Sample</i>	<i>NA</i>	<i>MCA</i>	<i>SSA</i>
CIN	1.0 x 10 ⁴	8.0x10 ³	4.1x10 ³
SP	1.4 x 10 ⁴	9.2x10 ³	6.1x10 ³

Key: CIN: Cabbage SP: Spinach, NA: Nutrient agar, MCA: MacConkey agar, SSA: Salmonella Shigella agar

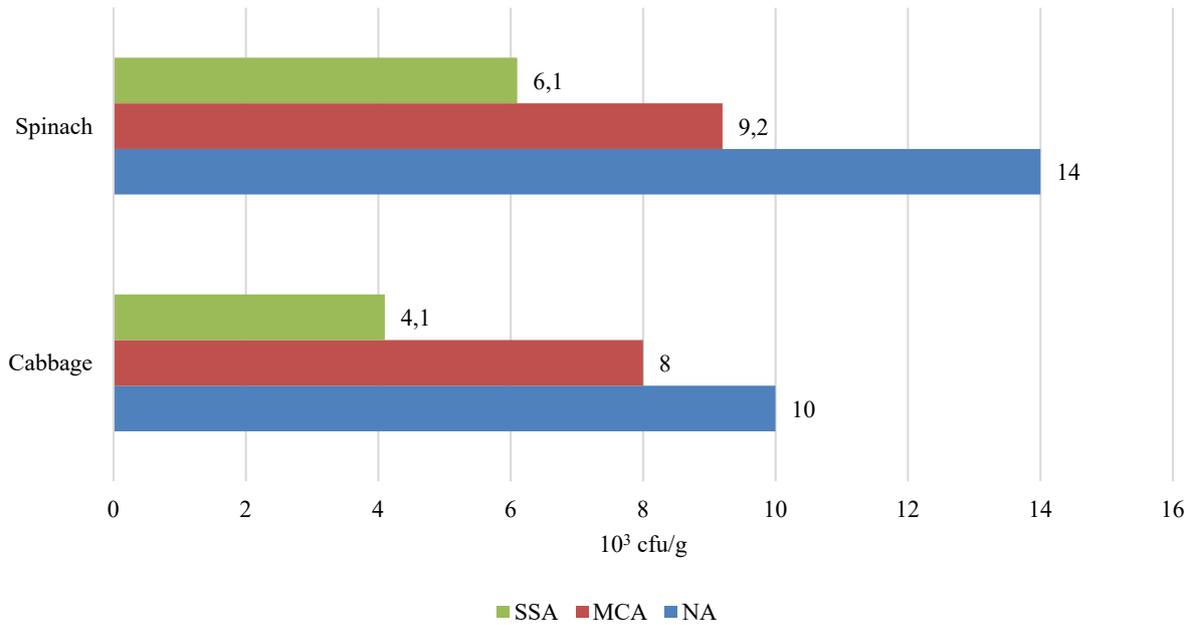


Figure 1: Chart Comparing the Average Bacterial Load from Spinach and Cabbage on the Different Agar used

From the percentage (%) occurrence (figure 2), it shows that *Salmonella* spp and *Escherichia coli* were the most predominant organisms isolated (23%), while *Streptococcus* spp was the least (8%).

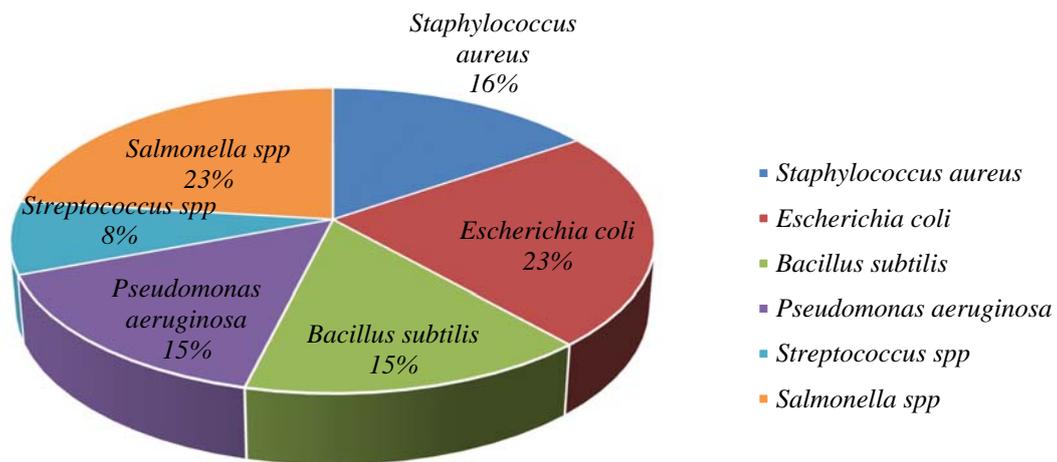


Figure 2: Chart showing the percentage (%) occurrence of the isolates in the samples

In this comparative assessment of the bacteriological content of cabbage and spinach, it was found that the level of bacteria in spinach is higher than that of cabbage. Generally, the bacteria found were similar but in different proportions (Table 3). Bacteria like *Staphylococcus aureus*, *Streptococcus* spp, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella* spp and *Escherichia coli* were found in the samples (Table 2).

Table 2: Showing the Cultural, Morphological and Biochemical characteristics of Bacteria Isolate from Spinach and Cabbage using MacConkey, Nutrient and Salmonella Shigella Agar

<i>Parameter</i>	<i>Isolate 1</i>	<i>Isolate 2</i>	<i>Isolate 3</i>	<i>Isolate 4</i>	<i>Isolate 5</i>	<i>Isolate 6</i>
Cultural characteristics	Dry, circular, Whitish to creamy flat on nutrient agar	Circular pink, elevated, on Mac Conkey agar	Whitish circular colonies on Nutrient agar	Transparent to milky irregular flat on Nutrient agar	Dry, spreading, whitish, flat on nutrient agar	Circular, pink colonies with black center on SSA
Morphological characteristics	Cocci in clusters	Long rods	Rod	Short rods	Cocci in chains	Rod
Gram reaction	+	-	+	-	+	-
Coagulase	+	-	-	ND	-	ND
Catalase	+	-	+	-	+	+
Indole	-	+	-	+	-	-
Oxidase	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Maltose	+	+	+	+	+	-
Lactose	-	+	-	-	+	+
Probable bacteria	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus</i> spp	<i>Salmonella</i> spp

Key :+ : positive --: negative **ND**: not determined

Table 3: Occurrence of the isolates in the samples

<i>Samples</i>	<i>Isolates</i>
Spinach	<i>Staphylococcus aureus</i> , <i>Escherichiacoli</i> , <i>Bacillus subtilis</i> , <i>Pseudomonasaeruginosa</i> , <i>Streptococcus</i> spp, <i>Salmonella</i> spp
Cabbage	<i>Staphylococcus aureus</i> , <i>Escherichiacoli</i> , <i>Bacillus subtilis</i> , <i>Pseudomonasaeruginosa</i> , <i>Salmonella</i> spp

Staphylococcus aureus is a gram positive bacterium found on the skin or in the nose of both healthy and unhealthy individuals. They come in contact with the vegetables during pre and post harvest practices like hand picking, planting, etc. also in the market where these vegetable are sold there is constant human contacts from both the vendors and consumers (Chaturvedi, *et al.*, 2013). Staphylococcal gastroenteritis is mainly caused by the consumption of food contaminated with *Staphylococcal aureus* strains (Izah *et al.*, 2016). The symptoms of staphylococcal gastroenteritis may include vomiting, abdominal cramps, headache, weakness and fatigue (Akhigbemidu *et al.*, 2015).

Salmonella spp and *Escherichia coli* could contaminate vegetables through fecal contamination of water, hands or /and soil. These pathogens could be from the water of irrigation and from the common unhygienic practices of the vendors. The microbial quality of irrigated water is critical because water contaminated with animal waste can introduce pathogens into vegetable products during pre-harvest and post-harvest activities via direct or indirect contamination. Therefore the bacteriological quality of irrigation water has a paramount importance to the safety of fresh and minimally processed vegetables (Solomon *et al.*, 2002). *Salmonella* and *Escherichia coli* cause varying degrees of intestinal disorders which include diarrhea which is sometimes bloody, urinary tract infection, abdominal cramps and dysentery (Odu and Imaku, 2013).

Bacillus subtilis is a gram-positive, rod-shaped facultatively anaerobic forming bacterium commonly found in soil and food due to preformed heat stable toxins. *Bacillus* in food products at concentrations exceeding 10^4 spores or vegetative cells per gram can cause food poisoning (Ehling-Schultz *et al.*, 2006; Meldrum *et al.*, 2009).

Pseudomonas aeruginosa comes in contact with vegetables through water, fertilizer or use of biocides during cultivation. The contamination of *Pseudomonasaeruginosa* on

vegetable may occur during harvesting, handling, processing and transit. Vegetable may come in contact with some soil, insects and water which they are represented as important sources of contamination in field including runoff water from nearby animal pasture and irrigation from contaminated sources (Chaturvedi, *et al.*, 2013).

4. Conclusion

Contaminated water, fecal materials, unhygienic environment and handling of vegetables and vegetable products by vendors were said to be the main source of contamination of fresh vegetable. The bacteria content of spinach was found to be more than that of the cabbage especially the inner layers of the cabbage because it is covered while spinach is exposed. This study therefore recommends that all those involved in the entire vegetable value chain should improve on their hygiene practice especially when handling or processing vegetables in order to prevent or reduce bacterial contamination to the barest minimum. Government should create portable water irrigation systems for vegetable farming, educate farmers, vendors and end users on the dangers of these pathogens so that more precaution will be taken from pre-planting to post harvesting stages. Also, consumers are advised to thoroughly wash vegetables during processing for consumption.

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