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COMPARATIVE ASSESMENT OF BACTERIOLOGICAL CONTENT OF CABBAGE AND SPINACH SOLD IN LAFIA.

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ABSTRACT

Most vegetables are usually green plants and are consumed raw or preheated. The comparative assessment of bacteria in spinach and cabbage were carried out to know the bacteria load of spinach and cabbage sold in Lafia Modern Market. Samples were bought from the market and taken to the Microbiology laboratory of Nasarawa State Polytechnic, Lafia for analysis. The samples were homogenized in a sterile beaker with peptone water for 15 minutes and serially diluted. Using pour plating method, the 1st and 3rd dilutions of each sample were cultured on Nutrient agar, MacConkey agar, and Salmonella Shigella agar and incubated at 37°C for 24 hours. The plate count indicated that on the average spinach had the highest bacteria count of 7.2x10³CFU/g, 4.6x10³CFU/g and 3.1x10³CFU/g on Nutrient agar, MacConkey agar and Salmonella Shigella agar respectively.

Introduction:

Vegetables are plants that can be consumed raw or pre-heated. Most vegetables are usually green plants e.g. spinach, lettuce, and pumpkin, while other vegetables that are non-green plants are cabbage, onion, mushroom, and radish. These vegetables are recommended to be consumed raw or pre-heated due to their fragility, hence they are not to be over cooked so their nutrients would not be lost (Chaturvedi, *et al.,* 2013).

The health benefits of fresh vegetables with high fiber and vitamins content make them more popular for the people who care about proper diet. The consumption of such products has increased in recent years. These vegetables have become

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Cabbage on the other hand has an average bacterial count of 3.0x10³CFU/g, 3.0x10³CFU/g and 1.3x10³ CFU/g on Nutrient agar, MacConkey agar and Salmonella Shigella agar respectively. The isolates identified are Staphylococcus spp (16%), Escherichia spp (23%), Bacillus spp(15%), Pseudomonas spp(15%), Streptococcus spp(8%) and Salmonella spp(23%). These isolated bacteria are pathogenic and are of public health importance due to their implication in food borne illnesses. It is recommended that farmers, vendors and consumers should improve on their hygiene when handling vegetables to prevent/reduce bacterial contamination

Keywords: *Cabbage, Spinach, vegetable, hygiene, Bacteriological.*

Vehicles for some kinds of pathogens when they are eaten raw (Chaturvedi, *et al.,* 2013).

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family amaranthaceae native to central and western Asia. Its leaves are eaten as vegetables. It grows most quickly in well-drained soil rich in organic matter such as compost manure and with a pH of 6.5 to 7. In order to grow spinach twice a year, plant it 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. Space plants 12 inches apart, this gives leaves room to reach full size (Ozlem and sener, 2005). Spinach is best known for being a rich source of iron. Spinach contains an array of micronutrients and phytochemicals. The major micronutrients in spinach are vitamins A (from β - Carotene), C K and folate, and minerals, calcium, iron and potassium. Other nutrients present in smaller quantities include vitamin E, some B vitamins – Thiamine (B_1), riboflavin (B_2) and the minerals magnesium, manganese and zinc (Athar *et al*., 2004).

Cabbage is an important vegetable known to mankind for over 4,000 years. 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 (Agrinfo, 2015). 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 22mg-150mg/100g. Its accumulated mineral source is at very high level of phosphorus, Sulfur, 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 dements. 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 inhabits 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.,* 2004).

These vegetables have become vehicles for some kinds of pathogens when they are eaten raw causing food poisoning. 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. 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 microbial load of cabbage and spinach sold in Lafia market.

Methods

Sample Collection

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

Sterilization of Equipments

All glass wares were properly washed and rinsed with clean water. An autoclave was used to sterilize the glass wares at 121^{0}C for 15minutes. The Inoculating or wire loop was sterilized using the flame from a Bunsen burner. The flaming of the wire loop was done repeatedly at the end of

every inoculation. A swab soaked in ethanol was used to sterilize the work bench.

Preparation of Agar

MacConkey agar, Nutrient agar and Salmonell Shigella agar were prepared according to the manufactures instruction, and were sterilized at 121^{0}C for 15 minutes.

Sample Preparation and Dilution

1 grams (1g) of each sample was weighed using a weighing balance, centrifuged and homogenized in 9ml of peptone water for 10mins. 1ml of the homogenate was then serially diluted (1:10) in five test tubes.

Enumeration of Bacterial Dilution

1ml of the aliquot was taken from 10^{-1} and 10^{-3} dilution and was spread onto the various agar (Nutrient agar, MacConkey agar and Salmonella Shigella agar) for determination of bacteria and was incubated at 37^{0}C for 24hours. The total number of colonies were counted and differentiated on the basic of their morphology and counts were made for different colonies.

Preparation of Pure Culture

Pure cultures of representative's bacteria colonies were obtained by cub-culturing and streaking onto sterile freshly prepared nutrient agar. The plates where then incubated at 37^{0}C for 24 hours.

Characterization of Isolate and identification

Identification of isolates was done using cultural and morphological and biochemical characteristics.

Results

Table 1: Total count of Bacteria Isolate in the samples on Nutrient Agar.

Sample	Dilution Factor	Number of colonies	Colony forming unit per gram(CFU/G)
Cabbage IN	10^1	7	$7.0x10^1$
	10^3	4	$4.0x10^3$
Cabbage OUT	10^1	26	$2.6x10^2$
	10^3	6	$6.0x10^3$
Spinach	10^1	40	$4.0x10^2$

	10^3	14	$1.4x10^4$

Table 2: Total Bacteria count in samples on MacConkey Agar

Sample	Dilution Factor	Number of colonies	Colony forming unit per gram(CFU/G)
Cabbage IN	10^1	8	$8.0x10^1$
	10^3	4	$4.0x10^3$
Cabbage OUT	10^1	14	$1.4x10^2$
	10^3	8	$8.0x10^3$
Spinach	10^1	28	$2.8x10^2$
	10^3	9	$9.0x10^3$

Table 3: Total bacteria count on Salmonella Shigella agar

Samples	Dilution Factor	Number of colonies	Colony forming unit per gram(CFU/G)
Cabbage IN	10^1	3	$3.0x10^1$
	10^3	1	$1.0x10^3$
Cabbage OUT	10^1	10	$1.4x10^2$
	10^3	4	$4.0x10^3$
Spinach	10^1	17	$1.7x10^2$
	10^3	6	$6.0x10^3$

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

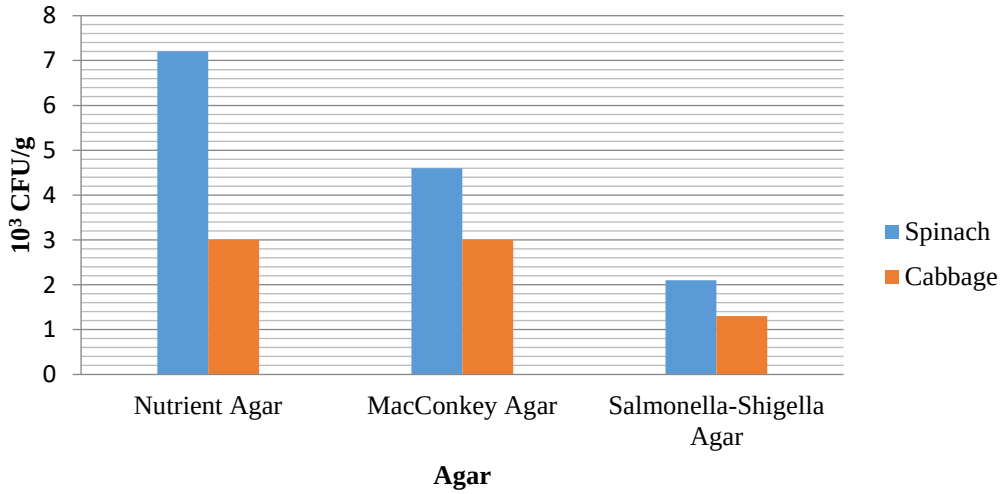

Table 4: Showing the Cultural , Morphological and Biochemical characteristics of Bacteria Isolate from Spinach and Cabbage using MacConkey, Nutrient and Salmonella Shigella Agar

Parameter	*Isolate 1*	*Isolate 2*	*Isolate 3*	*Isolate 4*	*Isolate 5*	*Isolate 6*
Cultural characteristic	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
Morphologicalcharacteristics	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	*Staphylococcus* spp	*Escherichia spp*	*Bacillus* spp	*Pseudomonas* spp	*Streptococcus* spp	*Salmonella spp*

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

Table 5.0: Occurrence of the isolates in the samples

Samples	*Isolates*
Spinach	*Staphylococcus*spp, *Escherichia*spp, *Bacillus*spp, *Pseudomonas*spp, *Streptococcus*spp, *Salmonella*spp
Cabbage	*Staphylococcus*spp, *Escherichia*spp, *Bacillus*spp, *Pseudomonas*spp, *Salmonella*spp

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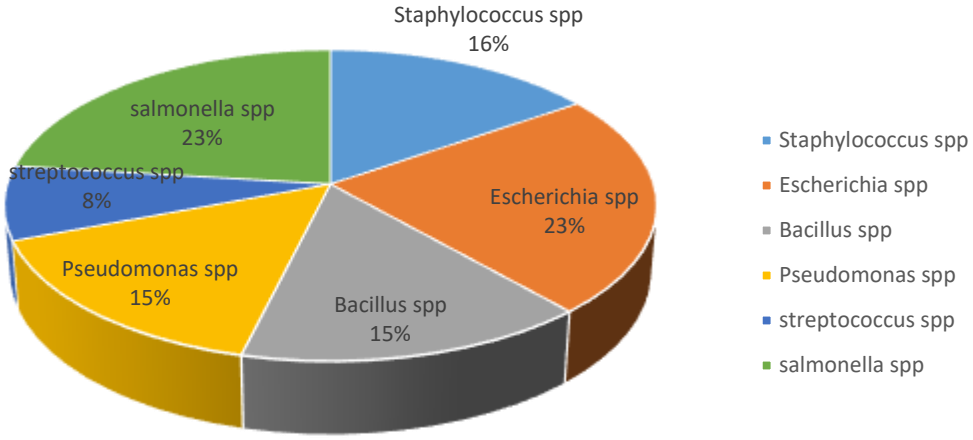

Figure 1: Chart showing the percentage (%) occurrence of the isolates in the samples

Discussion

Based on the bacteriological analysis carried out, the bacteria found are presented in table 5. Figure 1 shows the percentage (%) occurrence of the isolates in the samples. *Salmonella* spp 23%, *Escherichia* spp 23%, *Pseudomonas15*%, *Bacillus* 15%, *Staphylococcus* spp 16% and *Streptococcus* spp 8%. Bacteria count of isolates was taken from Nutrient agar, MacConkey agar and Salmonella Shigella agar as represented in table 1, table 2 and table 3 respectively. 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. Bacteria like *Staphylococcus* spp, *Streptococcus* spp, *Bacillus* spp, *Pseudomonas* spp, *Salmonella* spp and *Escherichia* spp were found in the samples.

Staphylococcus 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 and *Escherichia* contaminate vegetables through fecally contaminated 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 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 comes in contact with vegetables through water, fertilizer or use of biocides during cultivation. The contamination of pseudomonas 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)..

Conclusion

Contaminated water, fecal materials, unhygienic environment and handling of vegetable 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 government should create good water irrigation for farming, educate farmers, vendors and end users on the dangers of these pathogens so that more caution will be taken from pre-planting to post harvest. Also, consumers are advised to thoroughly wash and cook vegetables before consumption.

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