International Journal of Current Research and Review
DOI: https://doi.org/10.31782/IJCRR.2023.15502

Research Article

IJCRR
Section: Life Sciences

Effect of Apple Cider Vinegar on the Microbial Load of *Brassica Oleracea* (Cabbage) and *Lactuca Satira* (Lettuce) Available at Ilesan/ Sagamu Area of Ogun State, Nigeria

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ABSTRACT

Introduction: Vegetables like cabbage and lettuce which are widely consumed are important sources of micronutrients like vitamins and dietary fiber. These vegetables are prone to bacterial contamination as a result of pre- and post-harvest practices.

Aims and Objectives: Due to bacterial infestation of lettuce and cabbage in the course of usage, this work is aimed at employing safe chemical additives such as vinegar, at various concentration levels to investigate the microbial load of vegetables to ensure the safety of the vegetables for human consumption.

Methodology: A total of ten samples, cabbage (n=5) and lettuce (n=5) were purchased from Ilisan and Sagamu areas of Ogun state, Nigeria which lies within the latitude of6.8932°E and longitude of 3.7105°N.

Bacteriological quality of the samples was assessed by isolating and enumerating aerobic bacteria from the vegetable's samples upon the treatment with distilled water, 0.5% and 1% vinegar concentrations.

Results: A total of sixty isolates were recovered, twenty-six from cabbage and thirty-four from lettuce. Probable identities of the isolates were determined at the general level using standard methods. Micrococcus and staphylococcus recorded the highest percentage occurrence (16%) in cabbage, while *Bacillus* and *clostridium* had the highest percentage occurrence (12%) in lettuce. The treatment of the vegetables with 1% vinegar for five minutes resulted in highest significance ($p<0.05$) reduction of the microbial loads. There was an exposure time dependent reduction in the microbial load, favoring the higher exposure time. Additionally, the increase in the concentration of the vinegar from 0.5% to 1% also led to a reduction of the microbial load.

Conclusion: This study has shown that improperly washed vegetables could have poor microbial quality and vinegar treatment could offer first line control measure to enhancing food safety among consumers of cabbage and lettuce in Ilisan/Sagamu in particular, and globally in general.

Key Words: Food safety, Vegetables, Vinegar, Cabbage, Lettuce, Bacteria

INTRODUCTION

Raw and minimally processed fruits and vegetables are essential part of people's diet all around the world.[1] Cabbage and lettuce are one of the most consumed leafy vegetables.[2] The food and agricultural organization identified cabbage as one of the top twenty vegetables consumed globally and a great source of food globally.[3] Lettuce (*Lactuca sativa*) is a member of the family Asteraceae grown as a leafy vegetable used for salads, although it is also seen in other kinds of food, such as soups, sandwiches and wraps.[4]

Vegetable sanitization protocols recommend the use of chlorine, which have adverse effects such as cancer upon prolonged usage.[5] Vinegar is an interesting alternative to chlorine because it possess no risk to human health and is widely available in different forms such as apple cider vinegar or white distilled vinegar which are most commonly available.[6] Vinegar also does not have negative effect on the taste or appearance of these vegetables.[7] Vinegar contains acetic acid which is produced by fermentation of ethanol by acetic acid bacteria. Due to the presence of acetic acid in vinegar, a precursor in vinegar is known to possess antibacterial properties.[8]

Cabbage and lettuce are often consumed raw or minimally processed in salads, sandwiches and wraps. These vegetables are exposed to microbial contamination and potential

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ISSN: 2231-2196 (Print) **ISSN:** 0975-5241 (Online)

Received: 05.12.2022 **Revised:** 08.01.2023 **Accepted:** 06.02.2023 **Published:** 15.03.2023

pathogenic organisms by unsanitary cultivation and poor handling practices which threatens the health of consumers.[9] Examples of outbreaks related to fresh produce include cases of diarrhea and typhoid fever caused by *Escherichia Coli, Salmonella tophi* respectively.[10] Considering the widespread consumption of leaf vegetables like cabbage and lettuce, there isa need to ensure that they are bacteriologically safe by the adoption of natural and safer methods to ensure microbial food safety for the consumer and the community at large.[11] Consequently, this study is aimed at determining the effect of vinegar at 0.5% and 1% concentrations in reducing the microbial load in cabbage and lettuce vended in Ogun state, Nigeria.

MATERIALS AND METHODS

SAMPLING

A total of ten samples (n=10) consisting of five cabbage samples (n=5) and five lettuce samples (n=5) were purchased from major markets in Ilisan/Sagamu area of Ogun state, South Western Nigeria within the latitude of 6.8932 E and longitude 3.7105 N in the rain forest climatic region of the country. This was done between the period of January and February, 2020. All samples were kept in the packaging provided by the vendor and transported immediately to the laboratory for analyses.

ISOLATION

To ensure the integrity of the vinegar used for this study, 1ml was plated on nutrient agar (NA) and potato Dextrose agar (PDA) using pour plate method. The petri dishes were swirled and incubated aerobically at 37 C for 18 hours. No bacterial growth (NBG) was recorded. This procedure was also repeated at the end of the study, yielding the same result. 250ml solutions of 0.5% and 1% of Vinegar solution was prepared as well as distilled water. Subsequently, all materials were then sterilized using an autoclave for 15 minutes at 121°C. The cabbage ad lettuce was cut using a sterile knife and homogenized individually in a sterile mortar under aseptic conditions. To determine the initial microbial load present in the samples, 1g from the homogenized samples were serially diluted up to dilution 10^{-3}. An aliquot of 1ml each from the dilutions 10^{-1} and 10^{-3} were aseptically pour plated using nutrient agar. The petri dishes were swirled and incubated aerobically at 37°C for 18 hours.

25g of each sample was weighed out for each treatment which included:

- Treatment with water
- Treatment with 0.5% vinegar solution
- Treatment with 1% vinegar solution

The samples were soaked in the three-treatment listed above for 2.5 minutes and 5 minutes respectively. Thereafter, 1g from each treatment was measured out at the 2.5- and 5-minutes mark respectively and was serially diluted to 10^{-3}. An aliquot from each of the dilutions 10^{-1} and 10^{-3} were aseptically pour plated using nutrient agar. The petri dishes were swirled and incubated aerobically at 37°C for 18 hours.

SUBCULTURE OF ISOLATES

After 18 hours of incubation, visible colonies on the nutrient agar were counted and recorded as colony forming units per milliliter (cfu/mL). Representative colonies from each treatment were sub cultured on nutrient agar and were preserved after 24 hours in glycerol broth.

GRAM STAINING

Gram stain was used to differentiate between gram positive and gram-negative bacteria cells. This is dependent on their ability to retain the primary stain (crystal violet) when decolorized with acetone. The gram- negative bacteria retains the counter stain (safranin) and is visible under the microscope as pink or red while the gram-positive bacteria is purple or violet.

THE GRAM STAIN PROCEDURE

- A drop of distilled water was placed on a clean slide, and a sterile inoculating loop was used to pick a colony and make a smear.
- The smear was then gently heat fixed onto the slide over a flame.
- Crystal violet was applied for 30 seconds and then gently rinsed with water.
- Lugol's iodine was applied for 60 seconds and gently washed with water.
- The slide was decolorized using acetone, until the acetone runs clear.
- The counter stain (safranin) was applied for 30 seconds and gently rinsed with water.

The slide was then air dried and examined under the microscope with (X100) objective lens and immersion oil.[12]

CITRATE UTILIZATION TEST

All the isolates were streaked out on Simon citrate agar and incubated for 24 hours at 37°C. The result was observed and recorded after the stated time for color change from green to blue.[13]

STARCH HYDROLYSIS TEST

All the isolates were streaked out on starch agar prepared by the addition of soluble starch to nutrient agar and incubated for 24hours at 37°C. The plate was flooded with Lugol's iodine, and the result observed and recorded after the stated time for zones of cleaning around the isolate.[14,15,16]

INDOLE TEST

5ml of tryptophan broth was dispensed into test tube and sterilized for 15 minutes at 121°C. The isolates were inoculated into each test tube and incubated for 24 hours at 37°C. After the stated time, a few drops of Kovacs reagent were added to the test tube and results were observed and recorded.[17,18]

OXIDASE TEST

All isolates were smeared on clean glass slides and a few drops of tetra methyl- p- phenylenediamine reagent were applied. The result was observed and recorded accordingly.[19,20,21]

METHYL RED TEST

MR-VP broth was prepared using a solution of peptone water, dextrose sugar and diphosphate solution. This was dispensed into test tubes which were sterilized for 15 minutes at 121°C. The isolates were then inoculated into each test tubes which were sterilized for 15 minutes at 121°C. The isolates were then inoculated into each test tube and incubated at 121°C for 24 hours. After the stated time, a few drops of methyl red reagent were added, and the results observed and recorded accordingly.[22,23]

VOGES-PROSKAUER TEST

MR-VP Broth was prepared using a solution of Peptone water, dextrose sugar and diphosphate solution. This was dispensed into test tubes which were sterilized for 15 minutes at 121°C. The isolates were then inoculated into each test tube and incubated at 121°C for 48 hours. After the stated time, a few drops of KOH solution were added. After 30 minutes the results were observed and record accordingly.[24,25]

MACCONKEY TEST

All the isolates were streaked out on Macconkey agar and incubated for 24 hours at 37°C. The results were observed and recorded after the stated time.[26,27,28]

EOSIN METHYLENE BLUE (EMB) TEST

All the isolates were streaked out on Eosin methylene blue agar and incubated for 24 hours at 37°C. The results were observed and recorded after the stated time.[29,30]

RESULTS

Table 1: Results for the gram stain test, oxidase test, catalase test, citrate test, starch hydrolysis test, hemolysis test and probable organisms of theisolates.

Isolates	Gram Stain	Sample	Oxidase	Catalase	Citrate	Starch Hydrolysis	Hemolysis	Probable Organisms
1	-	L	-	+	+	+	γ	*Citrobacter, Erwina*
2	-	C	-	+	+	+	γ	*Neisseria*
3	+	C	+	+	-	-	γ	*Micrococcus staphylococcus*
4	+	C	+	+	+	-	β	*Micrococcus staphylococcus*
5	+	C	-	-	+	-	γ	*Enterococcus streptococcus*
6	+	L	+	+	+	-	γ	*Corynebacterium Lactobacillus*
7	-	L	+	+	+	+	β	*Vibro spp. Aeromanas, pseudome*
8	+	L	-	+	+	+	β	*Bacillus, Clostridium*
9	-	C	-	+	+	+	β	*Micrococcus staphylococcus*
10	-	C	-	+	+	-	β	*Neisseria*

Table 1: ***(Continued)***

Isolates	Gram Stain	Sample	Oxidase	Catalase	Citrate	Starch Hydrolysis	Hemolysis	Probable Organisms
11	-	C	-	+	+	-	β	*Citrobacter, Erwina*
12	+	C	-	+	+	+	β	*Actinomyces streptomyces*
13	+	L	+	+	+	+	β	*Actinomyces streptomyces*
14	-	L	+	+	+	+	γ	*Neisseria*
15	+	L	-	+	+	+	γ	*Bacillus, Clostridium*
16	+	L	-	+	+	-	γ	*Bacillus Clostridium*
17	-	C	-	-	+	+	β	*Neisseria*
18	-	L	-	-	+	+	β	*Vibrio spp. aeromona, Psedomanas*
19	+	L	+	+	+	+	β	*Bacillus, clostridium*
20	+	C	+	+	+	+	γ	*Micrococcus staphylococcus*
21	+	C	+	-	+	+	γ	*Enterococcus streptococcus*
22	-	L	+	+	-	+	γ	*Vibrio, Aeromona Pseudomonia*
23	+	L	-	+	+	+	γ	*Citrobacter, Erwina*
24	+	L	-	+	+	+	γ	*Actinomyces, Streptomyces*
25	-	C	-	+	+	+	β	*Neissera*
26	+	C	+	+	+	+	γ	*Micrococcus, staphylococcus*
27	+	L	+	+	+	+	γ	*Bacillus clostridium*
28	+	C	+	+	+	+	γ	*Micrococcus staphylococcus*
29	+	C	-	+	-	-	γ	*Micrococcus spp. staphylococcus*
30	-	L	-	+	+	+	β	*Vibrio spp. Aerp pseudomonas*
31	+	L	-	+	+	+	γ	*Bacillus spp. Clostridium spp.*
32	+	L	+	-	+	+	γ	*Bacillus spp. Clostridium spp.*
33	-	C	-	+	+	+	γ	*Neisseria spp*
34	-	C	-	+	+	+	β	*Neisseria spp*
35	+	L	-	+	+	+	γ	*Actinomyces spp. streptomyces spp.*
36	+	L	-	+	+	+	γ	*Bacillus spp. Clostridium spp.*
37	+	L	-	+	+	-	γ	*Actinomyces spp. streptomyces spp.*
38	+	L	+	+	+	+	γ	*Bacillus spp. Clostridium spp.*

Table 1: ***(Continued)***

Isolates	Gram Stain	Sample	Oxidase	Catalase	Citrate	Starch Hydrolysis	Hemolysis	Probable Organisms
39	+	C	-	+	-	+	γ	*Micrococcus spp. staphylococcus spp.*
40	+	C	+	+	-	+	γ	*Micrococcus spp. staphylococcus spp.*
41	-	L	-	+	+	+	β	*Vibrio spp, Aeromo Pseudomonas spp*
42	+	L	+	+	+	+	γ	*Bacillus, clostrid*
43	-	L	-	+	+	+	β	*Citrobacter, Erwina*
44	+	C	+	+	+	+	γ	*Micrococcus, staphylococcus*
45	-	L	-	+	+	+	β	*Vibrio, Aeronomas, Pseudomonas*
46	+	C	-	+	-	+	β	*Micrococcus, staphylococcus*
47	+	C	+	+	+	-	β	*Micrococcus, staphylococcus*
48	-	C	+	+	+	+	γ	*Neisseria*
49	+	L	+	+	+	+	γ	*Bacillus, clostridium*
50	+	C	+	+	-	-	β	*Micrococcus, staphylococcus*
51	+	L	-	+	-	-	β	*Actinomyces, Streptomyces*
52	-	C	+	+	-	-	γ	*Neisseria*
53	+	L	+	+	+	+	γ	*Actinomyces, Streptomyces*
54	+	L	+	+	+	+	β	*Actinomyces, Streptomyces*
55	-	C	+	-	+	+	γ	*Neisseria*
56	+	C	-	+	+	+	γ	*Micrococcus, staphylococcus*
57	+	L	-	+	+	+	γ	*Bacillus, clostridium*
58	-	C	+	+	-	+	γ	*Neisseria*
59	+	L	+	+	-	+	β	*Actinomyces, Streptomyces*
60	+	L	+	+	+	+	β	*Bacillus, clostridium*

KEY:

+ = Positive

- = Negative

L = Lettuce

C = Cabbage

Table 1 shows the presence of bacteria in the samples investigated. The lettuce and the cabbage samples show the presence of *Bacillus Clostridium, Micrococcus, Staphylococcus, Neisseria,Actinomyces, Streptomyces, Citrobacter, Erwina, Enterococcus, Streptococcus etc*. These research findings agree with the report of other researchers.[13,14,15] The presence of these organisms also indicates the possibility of fecal contamination and poor handling practices by vendors. [15] The occurrence of *Staphylococcus, Bacillus and Clostridium* in the samples is a major public health concern to consumers as they are responsible for food borne illnesses.[16]

Table 2: Results of Indole test, Methyl red test, VogesProskauer (VP) test, Eosin methyl blue (E MB) test and MacConkey (MAC) test on Gram- negative isolates.

ISOLATES	INDOLE	METHYL RED	VP	EMB	MAC
1	+	+	-	-	+
2	+	-	+	-	-
7	+	-	+	-	+
9	+	-	-	-	+
10	+	-	-	-	+
11	+	-	+	-	-
14	-	-	-	-	+
17	+	+	+	-	-
18	-	-	+	-	+
22	-	-	+	-	-
25	-	+	-	-	+
30	+	+	-	-	+
33	+	-	-	-	+
34	+	-	-	-	+
41	-	-	+	-	-
43	-	+	+	-	+
45	-	+	-	-	+
48	-	+	-	-	+
52	-	+	+	-	+
55	-	-	+	-	+
58	-	-	+	-	+

KEY:

+ = Positive

- = Negative

VP = Voges- Proskauer

EMB = Eosine Methylene blue

MAC = MacConkey

From table 2, it can be seen that Eosin methylene blue test all showed negative in all isolates evaluated. However, the Indole, methyl red and MacConkey test had a mixture of both positive and negative signs.

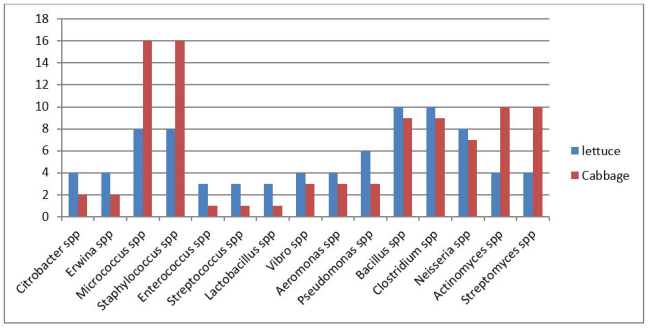

Figure 1: Percentage occurrence of bacteria in cabbage and lettuce samples.

As could be seen from figure 1, *Citrobacter spp.* appeared more in lettuce than in cabbage. In addition, *Erwina spp, Enterococcus spp, Streptococcus spp, Lactobacillus spp, Vibriospp, Aeromona spp, Pseudomonas spp, Bacillus spp, Clostridium spp, Neisseria spp,* appeared more in lettuce than in cabbage. On the other hand, *Micrococcus spp, Staphylococcus* featured more prominently in cabbage than in lettuce. This agrees with the findings of other researchers.[21]

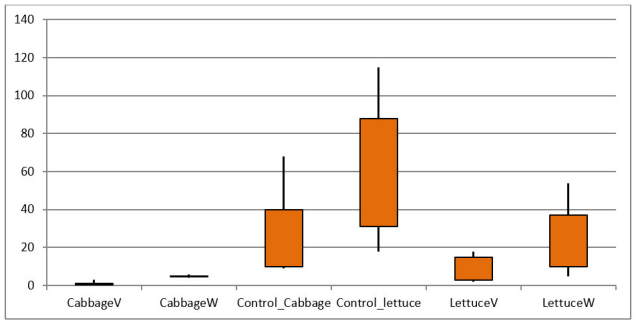

Figure 2: Boxplot representing the treatment of cabbage and lettuce with 0.5% Vinegar solution and water for 2.5 minutes.

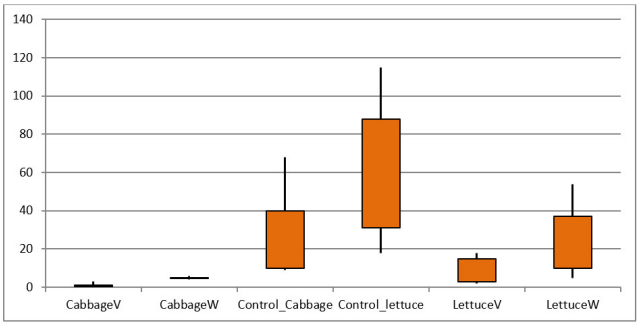

Figure 3: Boxplot representing the treatment of cabbage and lettuce with 0.5% vinegar solution and water for 5 minutes.

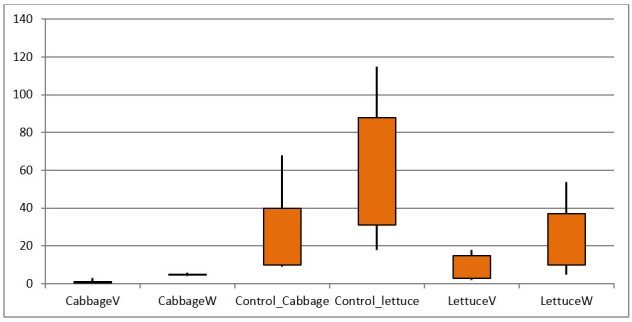

Figure 4: Boxplot representing the treatment of cabbage and lettuce with 1% vinegar solution and water for 2.5 minutes.

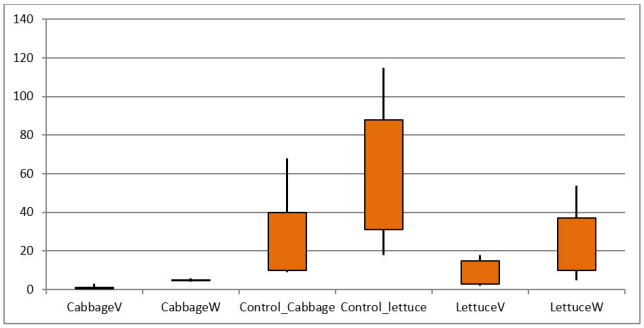

Figure 5: Boxplot representing the treatment of cabbage and lettuce with 1% vinegar solution and water for 5 minutes.

From figures 2 to 5, it can be seen that there is a significant reduction on the microbial load of cabbage and lettuce when the samples were treated with 0.5% and 1% concentration of vinegar respectively for five minutes. This is in agreement with research findings of other researchers.[22] This shows that the microbes underwent significant impairment following apple cider vinegar treatment which damaged the cell structural integrity- metabolic proteins and nuclear material thus reducing the microbial load.

DISCUSSION

Table 1 shows the presence of bacteria in the samples investigated. The lettuce and the cabbage samples show the presence of *Bacillus, Clostridium, Micrococcus, Staphylococcus, Neisseria, Actinomyces, Streptomyces, Citrobacter, Erwina, Enterococcus, Streptococcus* etc. These research findings agree with the report of other researchers.[13,14,15] The presence of these organisms also indicates the possibility of fecal contamination and poor handling practices by vendors[15]. The occurrence of *Staphylococcus, Bacillus* and *Clostridium* in the samples is a major public health concern to consumers as they are responsible for food borne illness.[16]

From table 2, it can be seen that Eosin methylene blue test all showed negative in all isolates evaluated. However, the Indole, methyl red and MacConkey test had a mixture of both positive and negative signs.

As could be seen from figure 1, *Citrobacter spp*. appeared more in lettuce than in cabbage. In addition, *Erwina spp, Enterococcus spp, Streptococcus spp, Lactobacillus spp, Vibriospp, Aeromonas spp, Pseudomonas spp, Bacillus spp, Clostridium spp, Neisseria spp,* appeared more in lettuce than in cabbage. On the other hand, *Micrococcus spp, Staphylococcus* featured more prominently in cabbage than in lettuce. This agrees with the findings of other researchers.[21]

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Findings from other researchers showed a reduction in bacterial load of lettuce and cabbage with a variation in concentration (5, 10, 15%). However, this work shows a better comparison economically since concentration as low as 1% can significantly reduce the microbial load and also preserve the organoleptic qualities of vegetables. The use of vinegar has thus been proven in this study to reduce the microbial load significantly at 0.5% and 1% for 5 minutes.

CONCLUSION

Pathogenic organism found in vegetables such as cabbage and lettuce can cause food borne disease. Hence, they constitute a huge public health risk to the consumers; the use of vinegar has thus been proven in this study to reduce the microbial load significantly at 0.5% and 1% for five minutes. Therefore, it is recommended that food handlers and consumers be properly educated on proper handling practices of vegetables to ensure their safety for consumption.

ACKNOWLEDGEMENTS

We acknowledge all who played one role or the other to the accomplishments of this research work. Specifically, to be mentioned, include Dr Ikenna Chukwu and Emmanuel Idika of Federal University of Technology, Owerri.

Source of funding: The authors funded the research work.

Conflict of Interest: No conflict of interest.

Authors' Contribution: Idika Digbo I, Ezekiel Chibundu N and Ekanem E.F- the above listed authors contributed equally in the course of this research work.

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