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Efficacy of vinegar as a disinfectant on the microbial quality of *Lactuca sativa* L.

Kubrat A. Oyinlola[1*], Gbemisola E. Ogunleye[2], Augustina I. Balogun[3] and K.I. Ayo-Abimbola[4]

[1]Department of Biological Sciences, Faculty of Applied Sciences, Koladaisi University, Ibadan, Oyo State. Nigeria. E-mail: bibimola.abiola@gmail.com; kubrat.oyinlola@koladaisiuniversity.edu.ng
[2]Department of Biological Sciences, Faculty of Applied Sciences, Koladaisi University, Ibadan, Oyo State. Nigeria. E-mail: gbemisola.ogunleye@koladaisiuniversity.edu.ng
[3]Department of Biological Sciences, Faculty of Applied Sciences, Koladaisi University, Ibadan, Oyo State. Nigeria. E-mail: augustina.balogun@koladaisiuniversity.edu.ng
[4]Department of Biological Sciences, Faculty of Applied Sciences, Koladaisi University, Ibadan, Oyo State. Nigeria. E-mail: kasope.ifeoluwa@gmail.com

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Abstract

Vegetables are widely exposed to microbial contamination, thus, harbor diverse microorganisms which may lead to infection outbreak. Microbial load on lettuce was estimated in this study, with vinegar, as a disinfecting agent. Randomly purchased samples were analyzed using standard microbiological methods to estimate microbial load in relation to disinfectant concentration and exposure time. Microbial load of samples rinsed with sterile distilled (control experiment) and tap water were 3.8 × 10^6CFU/g and 4.0 × 10^6 CFU/g, while those rinsed with vinegar ranged from 1.0 × 10^5 CFU/g to 2.7 × 10^6 CFU/g. Upon subjection to different exposure times (0, 5, 10 min) and vinegar concentrations (0.5%, 1%, 1.5%, and 2%), gradual reduction in microbial load was observed from 2.9 × 10^7 CFU/g, when rinsed with 0.5% vinegar to 1.0 × 10^6 CFU/g with 2.0% vinegar at initial time (0 min), at 5 min exposure, microbial load reduced from 1.9 × 10^7 CFU/g at 1.0% concentration to 3.0 × 10^5 CFU/g with 2.0% vinegar concentration, while the exposure of lettuce to 2.0% vinegar concentration for 10 min, showed no observable microbial growth. Lettuce samples analyzed were heavily contaminated with microorganisms, however, 2% vinegar at 10 min exposure time, was most effective at eliminating microbes. Healthy-looking vegetables may possibly harbor microorganisms, as such, good sanitary measures should be adopted before consumption.

Keywords: *Vegetable, Microbial contamination, Disinfection, Ready-to-eat, Vinegar, Food safety*

1. Introduction

Vegetables are generally a dietary source of nutrients, micronutrients and vitamins, thus, their increasing demand over the years. However, contamination of leafy vegetables, serving as vehicles of potential pathogens, such as *E. coli* O157: H7 or *Salmonella* spp. have frequently resulted in food disease outbreaks (Tomas-Callejas

* Corresponding author: Kubrat A. Oyinlola, Department of Biological Sciences, Faculty of Applied Sciences, Koladaisi University, Ibadan, Oyo State. Nigeria. E-mail: bibimola.abiola@gmail.com

et al., 2012). In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to such contamination (Amoah *et al*., 2009). Washing, the only procedure, aimed at cleaning and subsequently eliminating microbial, chemical and pest contamination, seemed not to be effective in total eradication, even though, reduction may be evident (Van Haute *et al*., 2013; Eni *et al*., 2010; WHO, 2015; and Olaimat and Holley, 2012). It is also important to note that the post-harvest washing water in most developing countries are not portable and could be a vehicle for microbial cross-contamination. Wash water, supplemented with chemical oxidants (chlorine, hydrogen peroxide, etc.), was proposed, even though, it's efficacy was reported to be hindered by the presence of organic matter in the wash water (Van Haute *et al*., 2013) and also, it's potential for the formation of byproducts such as trihalomethanes. Organic acids were alternative supplements, even though, researchers have stressed that supplementing wash water with disinfectants should be aimed at maintaining the water quality, to prevent cross contamination rather than having direct microbial effect on produce (Gil *et al*., 2009; and Davidson *et al*., 2013).

This study aimed at studying vinegar (acetic acid) as disinfectant, with varying concentrations and exposure times on microbial quality of lettuce.

2. Materials and methods

2.1. Sampling

Lettuce (*Lactuca sativa* L.) samples were purchased from vendors at Akinyele market, Akinyele Local Government, Oyo State. Nigeria. Samples were collected in sterile containers and transported to the Microbiology Laboratory, (Koladaisi University, Ibadan Nigeria) for immediate analysis. Vinegar (disinfecting agent) was also procured.

2.2. Microbial load determination

Initial washing was done with sterile distilled water, to eliminate sand particles and other dirts, after which, 10 g was rinsed in tap water, different vinegar concentrations (0.5%-2.0%), and sterile distilled water. Subsequent 10-fold serial dilutions (up to 10^{-8}) of each was carried out. 0.1 ml of 10^{-3}, 10^{-5} and 10^{-8} dilutions were introduced into petri dishes and sterile molten nutrient agar (Himedia, India) was added, swirled thoroughly to allow even distribution. The colonies were counted using a colony counter (Jiangsu Kangjian, China), after 24 h incubation at 37°C.

2.3. Effect of decontaminant concentration and exposure time on microbial load in lettuce

To determine the effect of different concentrations of vinegar and exposure time on lettuce samples, 50 g sample was weighed and washed in 450 ml of 0.5%, 1.0%, 1.5% and 2.0% vinegar solutions Aliquot (0.1 ml) of each rinse treatment (0.5%- 2.0% vinegar) at initial time of rinsing, then after 5 and 10 min exposure time was introduced into petri dish. Molten, sterile nutrient agar (Himedia, India) was poured, plates were swirled and allowed to set. Number of colonies on each plate was counted using a colony counter (Jangsu Kangjian, China) after 24 h incubation at 37ºC.

2.4. Isolation and identification of microorganisms

MacConkey Agar, Salmonella Shigella Agar, Potato Dextrose Agar and Nutrient Agar (Himedia, India) were inoculated with 0.1 ml diluents of each rinse treatment using the pour plate technique. The plates were allowed to solidify, inverted and incubated at 37ºC for 24 h. Distinct colonies were sub-cultured to achieve pure culture for further biochemical studies and identification of organisms.

Macroscopic, microscopic and biochemical characterization of selected microorganisms were conducted according to standard methods (Harrigan and McCance, 1966; Seeley and VanDemark, 1972; Olutiola *et al*., 2000) and were identified in reference to Bergey's Manual of Systematic Bacteriology (Sneath, 1986).

Colony count were converted into $\log_{10}$ CFU/g and mean values were calculated from the microbial evaluation of the lettuce sample.

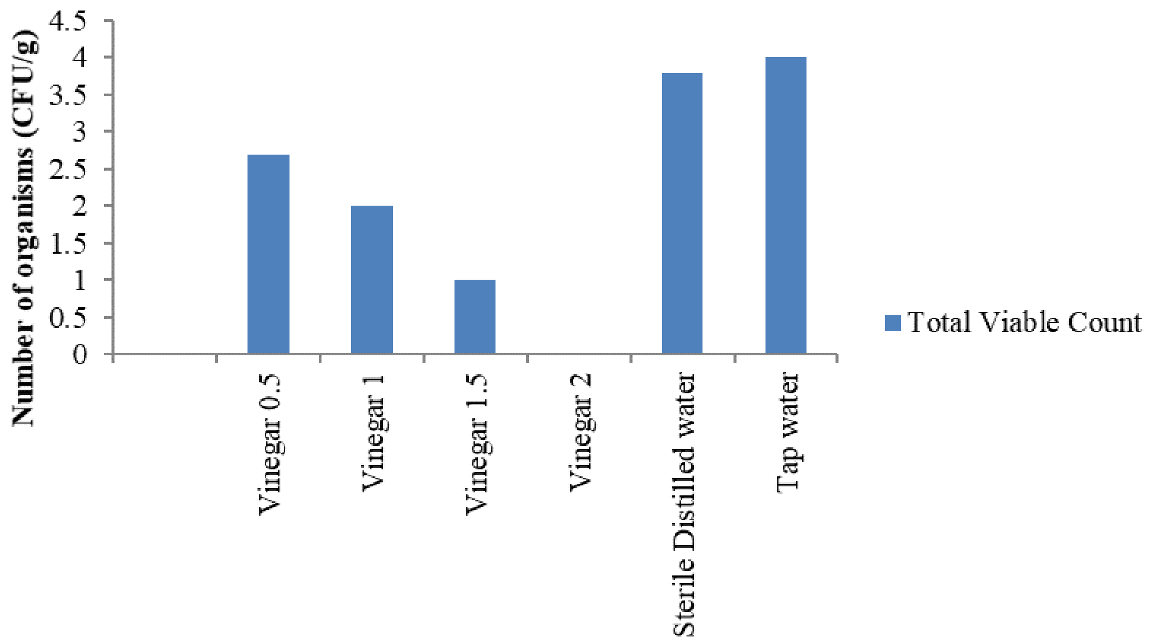

Figure 1: Mean total viable microbial count

3. Results

Most of the culture plates had observable growths showing that the lettuce was contaminated. Figure 1, depicting the microbial enumeration (total viable count) as well as quality of lettuce, showed that lettuce sample rinsed with sterile distilled water (control experiment) had 3.8×10^6 CFU / g while those rinsed with unsterile tap water had 4.0×10^6 CFU / g. Sample rinsed with the disinfectant showed the most reduced viable counts, ranging from 1.0×10^5 CFU / g to 2.7×10^6 CFU / g.

The effect of different vinegar concentrations and exposure times on the microbial load of lettuce sample was indicated in Table 1. At the initial time (0 min), a gradual reduction in microbial load was observed from 2.9×10^7 CFU / g, when rinsed with 0.5% vinegar concentration to 1.0×10^6 CFU / g of 2.0% vinegar concentration. After 5 min exposure, microbial load reduced from 1.9×10^7 CFU / g at 1.0% concentration to 3.0×10^5 CFU / g at 2.0% vinegar concentration. Exposure of lettuce samples to 2.0% vinegar concentration for 10 min, showed no observable microbial growth.

Table 1: Effect of concentration and exposure time of vinegar on lettuce sample

Vinegar concentration (%)	Mean microbial load (CFU/g)/Exposure time (min)		
	0	5	10
0.5	2.9×10^7	8.0×10^5	2.7×10^5
1.0	1.6×16^7	1.9×10^7	8.4×10^6
1.5	1.5×10^7	2.8×10^6	3.4×10^6
2.0	1.0×10^6	3.0×10^5	No growth

However, comparing the different vinegar concentrations, it was observed that 2.0% concentration had the most reduced number of microorganisms, irrespective of the exposure times.

Results of the biochemical characterization and identification of selected isolate, as shown in Tables 2 and 3, indicated the presence of six different probable bacteria species, from the thirty-nine selected isolates. They were *Bacillus* sp, *Staphylococcus* sp, *E. coli*, *Salmonella* sp., *Listeria* sp. and *Klebsiella* sp.

Table 2: Morphological characteristics of selected bacterial isolates

Isolates	Color	Margin	Opacity	Texture	Shape	Size	Elevation
LV1	Amber	Entire	Opaque	Shiny	Circular	Medium	Flat
LV2	Yellow	Entire	Opaque	Shiny	Circular	Medium	Flat
LV3	Cream	Entire	Opaque	Shiny	Circular	Small	Flat
LV4	Cream	Entire	Opaque	Shiny	Circular	Medium	Flat
LV5	Cream	Undulate	Opaque	Dry	Irregular	Medium	Flat
LV6	Yellow	Entire	Opaque	Shiny	Circular	Small	Flat
LV7	Cream	Entire	Opaque	Shiny	Circular	Small	Flat
LV8	Cream	Entire	Opaque	Dry	Circular	Small	Flat
LV9	Yellow	Entire	Opaque	Shiny	Circular	Small	Flat
LV10	Cream	Entire	Opaque	Shiny	Circular	Small	Flat
LV11	Cream	Undulate	Opaque	Dry	Irregular	Large	Flat
LV12	White	Filamentous	Translucent	Dry	Filamentous	Medium	Flat
LV13	Cream	Undulate	Opaque	Shiny	Circular	Medium	Flat
LV14	Yellow	Entire	Opaque	Shiny	Circular	Small	Flat
LV15	Cream	Entire	Opaque	Shiny	Circular	Small	Flat
LV16	Yellow	Entire	Opaque	Shiny	Circular	Small	Flat
LV17	Cream	Undulate	Opaque	Shiny	Irregular	Large	Flat
LV18	Cream	Undulate	Opaque	Dry	Irregular	Large	Flat
LV19	Cream	Undulate	Transparent	Dry	Irregular	Small	Flat
LV20	White	Filamentous	Translucent	Dry	Irregular	Medium	Flat
LV21	Cream	Entire	Opaque	Shiny	Circular	Medium	Flat
LV22	Cream	Entire	Opaque	Dry	Punctiform	Small	Flat
LV23	Yellow	Undulate	Opaque	Shiny	Circular	Medium	Flat
LV24	Cream	Entire	Opaque	Dry	Punctiform	Small	Flat
LV25	Blue	Entire	Opaque	Dry	Circular	Small	Flat
LV26	Cream	Entire	Opaque	Dry	Circular	Medium	Flat
LTW1	Cream	Undulate	Opaque	Shiny	Irregular	Large	Flat
LTW1	Cream	Undulate	Opaque	Shiny	Irregular	Large	Flat
LTW2	Cream	Entire	Opaque	Shiny	Circular	Small	Flat
LTW3	Cream	Undulate	Opaque	Shiny	Irregular	Medium	Flat
LTW4	Cream	Entire	Opaque	Shiny	Circular	Small	Flat

Table 2 (Cont.)

Isolates	Color	Margin	Opacity	Texture	Shape	Size	Elevation
LTW5	Yellow	Erose (Serrated)	Opaque	Shiny	Irregular	Large	Flat
LSDW1	Cream	Undulate	Opaque	Shiny	Irregular	Large	Flat
LSDW2	Cream	Filamentous	Translucent	Dry	Filamentous	Medium	Flat
LSDW3	Cream	Entire	Opaque	Shiny	Circular	Medium	Flat
LSDW4	Yellow	Undulate	Opaque	Shiny	Irregular	Large	Flat
LSDW5	Yellow	Entire	Opaque	Shiny	Circular	Small	Flat
LSDW6	Cream	Entire	Opaque	Shiny	Circular	Medium	Flat
LSDW7	Cream	Undulate	Opaque	Shiny	Circular	Large	Flat

Table 3: Biochemical characterization and probable identity of selected isolates

Isolates	Gram staining	Shape	Catalase	MR	VP	Indole	Oxidase	Motility	Urease	Citrate	Suc	Gala	Fru	Glu	Lac	Arab	Star	Probable organisms
LTW1	-	Rods	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	*Staphylococcus aureus*
LTW2	+	Rods	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	*Bacillus cereus*
LTW3	+	Rods	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	*Bacillus cereus*
LTW4	-	Rods	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
LTW5	+	Rods	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aurus*
LTW6	+	Rods	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
LTW7	+	Rods	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	*Listeria* sp
LTW8	+	Rods	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	*Listeria* sp
LTW9	+	Rods	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	*E.coli*
LTW11	+	Rods	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	*E.coli*
LTW13	+	Rods	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	*Bacillus cereus*
LTW14	-	Rods	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
LTW15	+	Rods	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	*Listeria* sp
LTW16	+	Rods	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	*Listeria* sp
LTW17	+	Rods	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	*E.coli*
LTW18	-	Rods	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	*E.coli*
LTW21	+	Rods	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococus aureus*
LTW26	+	Rods	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	*Klebsialla* sp
LSDW4	-	Rods	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	*Klebsiella* sp
LSDW7	-	Rods	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	*Salmonella* sp
LV23	+	Rods	+	+	-	-	-	-		+	+	+	+	+	+	+	+	*Salmonella* sp.

Note: - = Negative; += Positive; MR: Methyl Red; VP = Vogues Proskuer; SUC = Sucrose; Galac. = Galactose; Fruc = Fructose; Gluc = Glucose; Lact = Lactose; Arab = Arabinose; and Star = Starch

4. Discussion

The use of disinfectants in cleaning fruits and vegetables is one of the most important ways of preventing the transmission of diseases. Chlorine and its compounds are the most common types of disinfectants, nevertheless, there is a potential for the formation of byproducts such as trihalomethanes, thus, the use of alternative compounds.

From the results of this study, it was observed that the samples rinsed with vinegar had the least microbial count as against those with the tap and sterile distilled water. This further elaborate the use of vinegar as a better rinse agent than water (Atter *et al*., 2014). Rinse with tap water, showing highest count indicated that the tap water was not sterile enough for this purpose, thereby possibly adding to the microbial floral of the lettuce, since a reduction in count was observed in the batch rinsed with sterile distilled water (control experiment). These results confirmed previous reports of microbial load reduction observed in vegetables washed \ rinsed with disinfectants (Amoah *et al*., 2009; Eni *et al*., 2010; and De oliveira *et al*., 2012).

The presence of microorganisms in vegetables are a direct reflection of sanitary quality of cultivation, water, harvesting, transportation, storage and processing (Ray and Bhunia, 2007). Most of the organisms identified in this study have been isolated from different vegetables as reported in different studies (Uzeh *et al*, 2009; and Eni *et al*., 2010). Microbial count act as an indicator for food quality and shelf life (Pianetti *et al*., 2008) but many not necessarily relate to poisoning / infections. The high microbial count, estimated to as high as 10^6 CFU \ g may be a reflection of unhygienic practices from farm-to-fork. Similar values ranging between 10^5 to 10^7CFU / ml was reported by Eni *et al.* (2010) during the isolation of microbes from fruits and vegetables. Numerous other research studies reported high microbial content in vegetable (Abdullahi and Abdulkareem, 2010; Itohan *et al*., 2011; and Atter *et al*., 2014). However, according to HACCP-TQM technical guideline, raw foods containing less than 10^4 CFU / g, are regarded as "good", between 10^4 and 10^6 CFU / g as "average", 10^6 and 10^7 CFU / g as "poor" and any, containing greater than 10^7 CFU / g as "spoilt" (Anonymous, 1998). The values thus reported, makes the lettuce samples to be termed as being average and poor.

Increase in concentration and exposure time was found to be relevant in the role vinegar played as disinfectant to reduce microbial load. The current study revealed the efficacy of such, to reduce microbial load and it also showed a progressive decrease in microbial load with increasing vinegar concentration and exposure time. The result was in accordance with earlier reports (Eni *et al*., 2010; and Hashemi *et al*., 2017) that attributed such to reduction in pH which is unfavorable to most bacteria as well as prolonged continuous exposure. As such, vinegar could potentially be the simple, inexpensive disinfectant for most ready- to- eat vegetables, even though changes in taste had been reported (Eni *et al*., 2010) with such vegetables, this can be overcome by subsequent rinse in water. No spoilage sign was noticed on the lettuce in this study considering the reported microbial load, insinuating that outward appearance cannot be good criteria for analyzing microbial quality.

Probable organisms identified include *Bacillus* sp, *Staphylococcus* sp, *E. coli*, *Salmonella* sp. *and Klebsiella* sp., all of which were also reported to be isolated from fruits and vegetables (Eni *et al*., 2010). Ajayi *et al.* (2017) reported the presence of *Pseudomonas* sp, *Proteus sp, Enterobacter* sp, *Microroreus* sp, alongside the reported organism in this study.

Pseudomonas and Bacillus spp are part of plant microflora and are among the most common vegetable spoilage organisms (Vanderzant and Splittstoesser, 1992). *Staphylococcus, E. coli, Pseudomonas and Proteus* are also pathogenic and opportunistic bacteria of health concern. This further highlights the need to safeguard the health of the consumers by proper washing and decontamination of vegetables which are consumed without heat treatment.

5. Conclusion

This study showed that lettuce samples analyzed were heavily contaminated with microorganisms, but decontamination with an antimicrobial agent reduced the microbial load. Vinegar, at 2% concentration and for 10 min exposure, was more effective at eliminating microbes as against rinsing with tap water, which reflected an increase in microbial load, thus, suggesting that the water was actually not portable. It should also be noted that vegetables may look good and healthy, but still harbor microorganisms, as such, good sanitary measures should be adopted when handling ready-to-eat fruits and vegetables.

Conflicts of Interest

Authors declare no conflict of interest.

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