Efficiency of common washing treatments in reducing microbial levels on lettuce in Mali

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Abstract

Lettuce is one of the most cultivated vegetables (eaten raw) in Mali with a percentage of 69.4 amongst leafy vegetables and 30.4 of all vegetables. Vegetables consumed raw, particularly lettuce, are sources of foodborne pathogens. This study was conducted to assess bacterial contamination of unwashed lettuce and the efficiency of disinfecting it with tap water and varying concentrations of some chemical disinfectants: bleach (0.00285, 0.00570 and 0.00855 ppm), potassium permanganate (170, 340 and 510 ppm), vinegar (0.00285, 0.00570 and 0.00855 ppm) and common salt (500, 1000 and 1500 ppm) based on the three consecutive washing protocol recommended for vegetables in Mali. Lettuce samples were randomly selected from farms irrigated with untreated river water within communities five and six in Bamako. Lettuce leaves were exposed to low concentration of the chemical disinfectants at 15 min, intermediate at 10 min and high at 5 min. A bleach (0.00285 ppm)/vinegar (0.00285 ppm) combination disinfection was also tested at 15 min. Tap water, bleach, potassium permanganate, vinegar and common salt reduced faecal coliform populations by 1.3–2.9, 1.5–3.0 and 1.9–3.5 log CFU/100 g, at 5, 10 and 15 min, respectively. Disinfection treatment using bleach (0.00285 ppm) was found to be more effective than other disinfectants at all contact times. All the disinfectants reduced *Escherichia coli* populations by 2.0–2.8 log CFU/100 g on lettuce and completely eliminated *Salmonella* spp. at all treatment contact times. Disinfection treatment using bleach/vinegar combination at 15 min also reduced faecal coliform populations on lettuce by 2.8 log CFU/100 g and completely eliminated *E. coli* and *Salmonella* spp. All chemical disinfectants including tap water at 15 min reduced faecal coliform populations below the undesirable ICMSF (2011) level (1000 CFU/100 g wet weight). These could contribute to reduce the health risk associated with the consumption of lettuce in Mali.

Introduction

The consumption of raw salad is common in Francophone countries in West Africa including Mali (Bellwood-Howard et al., 2015; Coulibaly-Kalpy et al., 2017; Karg and Drechsel, 2018; Somda et al., 2019; Tounkara et al., 2019). Fast food in Bamako and its peri-urban areas have lettuce as one of its common ingredients (Karg and Drechsel, 2018). Most fresh lettuce used in Bamako is cultivated along drains and near open wells, where untreated irrigation water is commonly used (Traoré et al., 2013; Dia, 2017; Traoré et al., 2018, Traoré, 2020). In Bamako, these sources of irrigation water have been observed to have high faecal coliform contamination levels above the World Health Organization (WHO) recommended standards for unrestricted irrigation (Traoré, 2020). Apart from untreated water, manure and contaminated soils are also sources of faecal coliform contamination (Traoré, 2020) and are difficult to control.

Fortunately, consumers in Bamako widely practice washing of vegetables usually eaten raw before consumption (Traoré, 2020). Washing vegetables with clean running water is, however, not a common practice in Mali because only 41% of Malians have access to treated water (WHO, 2017a). Thus, vegetables are normally washed in a basin of standing water that has been contaminated through previous use, which could increase contamination levels instead of decreasing them (Kumwenda, 2019).

The disinfection methods for vegetables in Bamako vary widely and are applied ineffectively by vegetables consumers due to poor knowledge and inadequate information (Traoré, 2020). Records elsewhere, however, show that the efficacy of these methods could be improved by using the correct disinfectants at appropriate concentrations and contact times (Traoré et al., 2013; Van Haute et al., 2017; Woldetsadik et al., 2017; Subramanya et al., 2018). Vegetable consumers in Mali are, however, not aware of these appropriate methods of disinfection. Some of the disinfectants sold in Mali have been repackaged from their original containers, with no labels, while others have foreign labels, are expired or are counterfeit. In addition, these disinfectants are also mostly exposed to ultraviolet radiation in the open market, which affects the quality and efficacy. The Mali Health Service (MHS) has prescribed the following protocol for disinfecting vegetables which are eaten raw: washing with tap water, followed by immersion in either bleach (NaClO) or potassium permanganate ($KMnO_4$) solutions at 0.00285 ppm and 170 ppm for 15 min respectively, and a final rinse in tap water (MHS, 2005). Majority of Malians are, however, not aware of this protocol, and this disinfection method has also been reported as ineffective for complete disinfection of lettuce (Traoré et al., 2013). The efficiency of common vegetable disinfectants in Mali (bleach, potassium permanganate, vinegar (acetic acid CH_3COOH), common salt (NaCl) and tap water), in reducing faecal coliforms such as *Escherichia coli* and *Salmonella* spp. on irrigated lettuce from Bamako were thus evaluated in this study.

Materials and Methods

Lettuce sampling procedure

The research was carried out in three lettuce farms with an average size of 0.25 hectare irrigated with untreated river water, in each of communities five and six within Bamako. Fifteen heads of lettuce, were collected at random per farm. The samples were then placed in sterile polythene bags and transported on ice to the laboratory where they were analysed immediately for bacterial contamination before and after disinfection or stored at 4ºC and used within 24 h after harvest. Lettuce leaves were removed from each head and combined to obtain a composite sample for each farm (Hayes, 1995).

Preparation of disinfectant solutions

Five commonly used vegetable disinfectants in Mali: "eau de javel" (bleach) manufactured by Alvejante (Ivory Coast), potassium permanganate (Cooper France), vinegar (Vinaigres Fuchs Sas, France), common salt (NaCl) and tap water were used in this study. Three concentrations of the four chemical disinfectants (lower, intermediate and higher concentration) were prepared as follows using sterile distilled water: bleach at 0.00285, 0.00570 and 0.00855 parts per million (ppm); potassium permanganate at 170, 340 and 510 ppm; vinegar at 0.00285, 0.00570 and 0.00855 ppm and common salt at 500, 1000 and 1500 ppm. The three concentrations of bleach, potassium permanganate and vinegar were chosen based on the two recommended vegetable disinfectants in Mali (MHS, 2005), while those of the common salt were based on estimates from the results of previous study by Traoré (2020). Two litres of each disinfection solution including tap water were poured in a clean (washed with household soap) plastic container.

Disinfection procedure

The disinfection method tested in this study was based on three consecutive washing protocol recommended for vegetables in

Mali (MHS, 2005). Hundred grams of each composite lettuce leaves from each farm were washed in two litres tap water for one minute, followed by immediate immersions in respective disinfection solutions at specific contact time depending on the concentration and finally rinsed in two litres tap water for one minute. The high, intermediate and low concentration of disinfection solutions were exposed for 5, 10 and 15 min, respectively. Disinfection with tap water was equally tested at 5, 10 and 15 min. Faecal coliform populations colony-forming unit/100 g (CFU/100 g) in each lettuce sample as well as qualitative assessment for *Salmonella* spp. were determined before (unwashed) and immediately after disinfection. All disinfection procedures were done in LaboREM-Biotech, Faculty of Sciences and Techniques, University of Sciences, Techniques and Technologies of Bamako at approximately 25 ± 3°C.

Faecal coliform populations were determined by the method of NFV 08 060 (2009). This consisted of weighing 100 g of lettuce (crushed) in 1000 ml of sterile buffered saline solution and shaken vigorously for two minutes. A serial dilution was performed in tubes containing the buffered solution and two copies of the broth inoculated from each dilution on Petri dish containing Eosin Methylene Blue media and incubated at 44.5°C for 24–48 h. The number of colonies were counted in each Petri dish and the number of bacteria evaluated as CFU/100 g of sample.

Escherichia coli populations was determined by the method of ISO 16649-2 (2019) and *Salmonella* spp. by the method of ISO 6579-1 (2017). The method of isolation of pure cultures described in Bergey's manual of systematic bacteriology (Holt, 1986) for the isolation of pure cultures from a mixture of species were strictly followed. *Escherichia coli* and *Salmonella* spp. cultures from different samples were used in this study. Positive cultures of Tryptone Bile X-glucoronide (blue colonies) were selected for estimating populations of *E. coli* in lettuce samples. In the case of identification of *Salmonella* spp., the mother suspension was incubated for 24 h at 37ºC, and then one millilitre of the solution was put into 10 ml of the sterile Rappaport-Vassiliadis *Salmonella* Enrichment Broth prepared and incubated for 24 h. Subsequent to the second incubation, 100 µl of the solution was seeded on Hektoen agar medium (HEKT-D) and incubated for another 24 h. The positive colonies on HEKT-D (white colonies with black centers) were seeded on Triple Sugar Iron agar medium. From each positive culture of *E. coli* or *Salmonella*, the isolates were purified by selecting a colony and leaving streaks on the selective medium at least three times and incubating the dish at 44ºC for *E. coli* and 37ºC for *Salmonella* for 24–48 h. The purity of *E. coli* and *Salmonella* spp. colonies was confirmed after incubation of their respective dishes by Gram staining. After confirmation of the Gram reaction, a few colonies were mixed in five millilitres of a sterile buffered saline solution, and the suspension distributed in the tanks of the Analytical Profile Index (API 20E) gallery and incubated for 24 h for biochemical confirmation. The results were interpreted using the catalogue and confirmed by the identification software.

Following the results obtained from this experiment, bleach (0.00285 ppm)/vinegar (0.00285 ppm) combination disinfection was also tested at 15 min using the same protocol above. The entire experiment was repeated three times with fresh composite lettuce samples (from the same study area in Bamako) for the second and third repetitions. The mean faecal coliform populations on lettuce before disinfection for the three experiments was 4.5 log CFU/100 g and its corresponding *E. coli* populations were 2.8 CFU/100 g. *Salmonella* spp. was present in lettuce samples in all experiments before disinfection.

Data handling and analysis

The reduction of faecal coliforms populations (CFU/100 g) on each lettuce sample after disinfection relative to unwashed lettuce was calculated. Faecal coliform and *E. coli* populations data were log-transformed and subjected to ANOVA. Where statistical difference was found, means were separated using Turkey's HSD test ($P < 0.05$). All analyses were conducted in GenStat 11th Edition.

Results

Efficiency of common disinfection methods on faecal coliform level on lettuce irrigated with untreated river water from farms in Bamako

Two-way ANOVA for mean faecal coliform populations showed no interaction between contact time and disinfectant ($F_{(8, 44)}$, $P = 0.988$), however, individual effects were significant (contact time, $F_{(2, 44)}$, $P < 0.001$; disinfectant $F_{(4, 44)}$, $P < 0.001$).

At all contact times, the mean log reduction of faecal coliform populations on lettuce did not vary amongst bleach, potassium permanganate, vinegar and common salt regardless of the concentration and ranged between 2.4–3.5 log CFU/100 g (Table 1). Mean faecal coliform populations reduction in tap water at 15 min (1.9 log CFU/100 g) was not significantly different from all disinfectants at all contact

TABLE 1

Faecal coliform populations and log reduction on lettuce cultivated using untreated water from river in Bamako and washed with some vegetable disinfectants at different contact

Contact time (min)	Vegetable disinfectant (ppm)	Mean faecal coliforms (CFU/100 g)	Mean log reduction of faecal coliforms (CFU/100 g)
5	Unwashed	4.5	-
	Tap water	3.2^{c}	1.3^{c}
	NaClO (0.00855)	2.0^{abc}	2.5^{abcd}
	$KMnO_4$ (510)	1.6^{abc}	2.9^{ab}
	Vinegar (0.00855)	2.1^{abc}	2.4^{abc}
	NaCl (1500)	2.1^{abc}	2.4^{abc}
10	Tap water	2.9^{bc}	1.6^{bc}
	NaClO (0.00570)	1.7^{abc}	2.8^{abc}
	$KMnO_4$ (340)	1.5^{ab}	3.0^{ab}
	Vinegar (0.00570)	1.8^{abc}	2.7^{abc}
	NaCl (1000)	1.7^{abc}	2.8^{ab}c
15	Tap water	2.6^{abc}	1.9^{abc}
	NaClO (0.00285)	1.0^{a}	3.5^{a}
	$KMnO_4$ (170)	1.2^{a}	3.3^{a}
	Vinegar (0.00285)	1.3^{ab}	3.2^{a}
	NaCl (500)	1.1^{a}	3.4^{a}
	NaClO (0.00143)/Vinegar (0.00143)	1.7^{abc}	2.8^{abc}

Means within column followed with different letter are significantly different (Turkey's HSD test $P < 0.05$)

times. Mean faecal coliform populations reduction in bleach (0.00285 ppm), salt (500 ppm), potassium permanganate (170 ppm) and vinegar (0.00285 ppm) at 15 min was significantly higher than tap water disinfection at 5 and 10 min. The log reductions of faecal coliform population in tap water at 5, 10 and 15 min ranged from 1.3–1.9 log CFU/100 g and did not differ significantly ($P < 0.05$). Bleach/vinegar combination (0.00143 ppm/0.00143 ppm) applied for 15 min reduced faecal coliform populations by 2.8 log CFU/100 g.

Efficiency of common disinfection methods on E. coli populations on lettuce irrigated with river water from farms in Bamako

Two-way ANOVA for mean *E. coli* populations showed that main effects (contact time ($F_{(2, 44)}$, $P < 0.001$); and disinfectant ($F_{(4, 44)}$, $P = 1.000$) as well as their interaction (contact time × disinfectant ($F_{(8, 44)}$, $P = 1.000$) were not significantly different. *Escherichia coli* populations after disinfection ranged between 2.0–2.1 log CFU/100 g (Table 2). Bleach/vinegar combination completely eliminated *E. coli* populations on lettuce samples at 15 min.

Efficiency of common disinfection methods on Salmonella spp. on lettuce irrigated with river water from farms in Bamako

There was complete disinfection of *Salmonella* spp. on lettuce samples with disinfectants at all contact times.

Discussion

All disinfectants tested in this study reduced faecal coliform populations on lettuce, but could not completely eliminate them. This finding is in agreement with those of several other researchers where the use of similar disinfectants even at higher concentrations

TABLE 2

E. coli populations and reduction on lettuce cultivated using untreated water from river in Bamako and washed with some vegetable disinfectants at different contact

Contact time (min)	Vegetable disinfectant (ppm)	Mean *E. coli* populations (CFU/100 g)	Mean reduction of *E. coli* populations (CFU/100 g)
5	Unwashed	2.8	-
	Tap water	0.8	2.0
	NaClO (0.00855)	0.7	2.1
	$KMnO_4$ (510)	0.7	2.1
	Vinegar (0.00855)	0.7	2.1
	NaCl (1500)	0.7	2.1
10	Tap water	0.7	2.1
	NaClO (0.00570)	0.7	2.1
	$KMnO_4$ (340)	0.7	2.1
	Vinegar (0.00570)	0.8	2.0
	NaCl (1000)	0.8	2.0
15	Tap water	0.7	2.1
	NaClO (0.00285)	0.7	2.1
	$KMnO_4$ (170)	0.7	2.1
	Vinegar (0.00285)	0.7	2.1
	NaCl (500)	0.7	2.1
	NaClO (0.00143)/Vinegar (0.00143)	0.0	2.8

No significant difference ($P > 0.05$) was found amongst disinfectants at all contact times could not completely eliminate faecal coliform on lettuce and other vegetables (Pan and Nakano, 2014; Petri et al., 2015; Van Haute et al., 2017; Woldetsadik et al., 2017; Bencardino et al., 2018; Bhilwadikar et al., 2019; Pourzamani et al., 2019). The high log reductions in faecal coliform populations obtained with some of the disinfectants, particularly, bleach and potassium permanganate (2.5–3.5 log CFU/100 g) could be due to the three consecutive washing system used in the present study. Disinfection with tap water at 15 min was as effective as the other sanitisers at all contact times. Clean water is well known to flash out some microorganisms from vegetables (Pan and Nakano, 2014; Dao et al., 2018; Subramanya et al., 2018; Bhilwadikar et al., 2019). For instance, Subramanya et al. (2018) demonstrated a significant reduction of the faecal coliform populations on vegetables after three consecutive washing in sterile distilled water (1.6×10^7–5.8×10^3 CFU/ml). Almost 100-fold reduction was even achieved following the first wash. Additionally, tap water contains chlorine with concentrations of 0.2–1.0 mg/l (WHO, 2003) which could also disinfect microorganisms.

Nevertheless, complete elimination of microorganisms on raw vegetables is desired. This, therefore, could require increasing the concentration or contact time. However, increasing the concentration or contact time in other studies affected the quality, shelf-life and chemical content of vegetables (Botondi et al., 2015; Park and Kim, 2015; Rodoni et al., 2015; Bridges et al., 2018; Brodowska et al., 2018; Lee et al., 2018; Bhilwadikar et al., 2019; Gehringer and Kaletunç, 2020). In the present study, potassium permanganate at the lowest concentration (recommended concentration) caused purpled discoloration on the middle portion of the lettuce leaves. This discoloration was also observed on entire young leaves in the highest concentration. These reasons, coupled with its scarcity in Mali could explain why only 3–11% of vegetable consumers used this disinfectant compared to bleach (73–87%) which is readily available (Traoré, 2020). On the contrary, none of the tested concentrations of bleach or remaining disinfectants affected the physical quality of lettuce in the study. Traoré et al. (2013) using 2.6 mg/l of bleach for 15 min at almost a 1000-fold increase of the lowest concentration in this study, obtained complete disinfection of lettuce which did not affect its quality and the chlorine residues on the produce was less than the maximum acceptable value (5 mg/l) in drinking water (WHO, 2017b). Therefore, it would be worthwhile investigating the efficacy of this high concentration on a larger scale by consumers using the disinfection procedure employed in this study.

Vinegar and sodium chloride also had at least 2 log reductions in faecal coliforms populations, which is in agreement with findings of Amoah et al. (2007) and Woldetsadik et al. (2017). Between these aforementioned disinfectants, sodium chloride (salt) is less expensive, safer and readily available, so further studies should consider varying the highest tested concentration and contact time to determine if complete disinfection can be obtained.

The combination of bleach and vinegar at low concentrations (0.00143 ppm/0.00143 ppm) applied for 15 min reduced faecal coliform populations and completely eliminated *E. coli* and *Salmonella* spp. on lettuce. These results suggest that it is possible to have complete disinfection with a slightly higher concentration of this disinfectant mixture.

However, several authors (Chauhan et al., 2008; O'Malley et al., 2019; Morim and Guldner, 2020) have highlighted the dangers in combining bleach and vinegar as disinfectant even as a surface cleanser. Combining bleach and vinegar produces chlorine gas, an asphyxiant, which upon inhalation at low concentrations (1–3 ppm), causes difficulties in breathing, irritation of oral mucosa and eyes, while exposures at higher concentrations (≥ 430 ppm) can cause death within 30 min (Chauhan et al., 2008; Morim and Guldner, 2020).

It can be inferred from this study that disinfection of lettuce before consumption could be a crucial point for health risk reduction in Mali. The suggestion of Murray et al. (2017) for multiple intervention approach from production of vegetable prior to consumption could be the most effective in preventing vegetable contamination compared to sole disinfectants. In Mali, the main sources of microbial contamination on vegetables are from the farms, with irrigation water, soil and manure contributing the largest (Samaké et al., 2011; Traoré et al., 2013; Dia, 2017; Traoré, 2020). Controlling these sources of contamination is difficult in the short to medium term for municipal authorities and vegetable producers due to high cost. Treating irrigation water with disinfectants (particularly bleach) in addition to disinfection of lettuce prior to consumption could be critical in reducing the potential risks associated with its consumption and should therefore be prioritised.

Conclusions

All chemical disinfectants including tap water at 15 min reduced faecal coliform populations below the undesirable ICMSF (2011) level (1000 CFU/100 g wet weight). The low, intermediary and high concentrations of all chemical disinfectants (bleach, potassium permanganate, vinegar, common salt and bleach/vinegar) applied for 5, 10 and 15 min respectively, reduced faecal coliform populations on lettuce by 2.4–2.9, 2.7–3.0 and 2.8–3.5 log CFU/100 g. Reductions in faecal coliform populations on lettuce with tap water were 1.3, 1.6 and 1.9 log CFU/100 g at 5, 10 at 15 min respectively. Reductions in faecal coliform populations on lettuce with tap water at 15 min were similar to the chemical disinfectants. All chemical disinfectants and tap water, reduced *E. coli* populations on lettuce and completely eliminated *Salmonella* spp. on lettuce regardless of the concentration and contact time. Bleach/vinegar combination at 15 min reduced faecal coliform populations on lettuce by 2.8 log CFU/100 g and completely eliminated *E. coli* and *Salmonella* spp.

Recommendations

MHS should investigate the efficacy of their two recommended vegetable disinfectant, particularly bleach at higher concentration and on a larger scale. The disinfection methods tested in this study should be evaluated by food vendors and restaurateurs to determine its efficacy on lettuce and other raw eaten vegetables on a large scale. Sensory analysis following the disinfection methods should also be done.

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