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EFFICACY OF SOME SANITIZING AGENTS ON *SALMONELLA SPECIES* DETECTED IN RINSED WATER OF VEGETABLES EATEN RAW IN SAMARU, ZARIA

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ABSTRACT

Food-borne infection is one of the major disease outbreaks in developing countries most especially those cause by enteric pathogens like *Salmonella species*. The study was set out to examine the effects of vinegar, common salt and potash as sanitizers at different concentrations and exposure time on *Salmonella species*. Samples used include; carrots (10), cabbage (10) and lettuce (10). The results indicated that out of the thirty (30) samples collected, *Salmonella typhi* were isolated from 4 carrot, 2 cabbage and 3 lettuce samples. While 1,3 and 2 are from carrot, cabbage and lettuce samples respectively yielded other types of unidentified *Salmonella species*. The number of colonies formed by *Salmonella species* significantly decrease with increase in vinegar concentration and exposure time. Vinegar was found to be the most potent sanitizer followed by common salt while potash (white) had the slowest effect on *Salmonella species.* Therefore, washing vegetables with vinegar solution is advisable because it helps in minimizing potential health risk that could affect eating raw vegetables since the use of potable water for vegetable farming is difficult, even though is strongly suitable. Nevertheless, there is clear need of enlightenment to vegetable sellers on post-harvest handling, purposely to cut down the degree of contamination.

INTRODUCTION

Vegetable can be described as plants with edible parts especially leafy or fleshy parts that are used mainly for soups or salads or to accompany main courses. They serve as an extraordinary dietary source of nutrient, micronutrient, vitamins and fibre for humans and this is vital for health and well-being.

Vegetable and other farm products are widely expose to microbial contamination through contact with dust and water, by handling at harvest or post-harvest processing. Potential pre-harvest contamination sources of microorganisms include soil, in adequately composted manure, faeces, irrigation water and human handling. Vegetables irrigated with waste water have pathogens isolated on such crops after harvesting (Ensink *et al*., 2007). Post-harvest sources include human handling, harvesting equipment, transport containers wild and domestic animals, insects, dust, transport vehicles and processing equipment (Beuchat, 2002).

Difference in microbial profiles of various vegetable, results largely from unrelated factors, such as resident microflora in soil, manures, sewage or irrigation water, transportation and handling by individual retailers. Continued use of the untreated waste water and manure as

fertilizer for the production of vegetables in developing countries is a major contributing factor to contamination (Amoah *et al.*, 2009). Outbreak of human infections associated with the consumption of fresh or minimally processed vegetables have increased in recent years despite their nutritional health benefit (Beuchat, 1996 and Beuchat, 2002). Enteric pathogens such as *Escherichia coli* and *Salmonella species* are among the greatest concerns during food related outbreaks.

Vegetables free from bacteria are essential to minimize the health benefit promised by adequate consumption of the produce. Proper washing of vegetable is essential for decontamination. Treatment of produces with chlorinated water reduces populations of pathogenic and other microorganism on fresh produce. Also water supplement with varying concentrations of organic acids has been shown to reduce microbial population on vegetables (*Beuchat* 1998).

MATERIALS AND METHOD

Sample collection

Thirty (30) different samples of fresh vegetables were collected from open market in Samaru Zaria. The samples are: 10 cabbages (*Brassica oleraces*), 10 carrots (*Daucus carota*) and 10 lettuces (*Lactuca sativa*). The samples were collected aseptically inside the polythene, in the morning just after delivery of the vegetables, transported straight to the laboratory for processing. All samples were purchased from different retailers at different points in the market.

Preparation of Media

All media used were prepared in the laboratory according to the manufacturer's instructions and were dispensed into sterile Petri dishes, universal containers and test tubes in amount required. All prepared media were refrigrated for preservation.

Sample Processing

Each of the samples collected were sliced ready to eat salad vegetables and then 25 g of each sample was weighed and washed in 225 ml of distilled water. The rinsed water of each vegetable sample was transferred into sterile beaker for the purpose of *Salmonella* isolation (Food and Agricultural Organisation, 1992).

Enrichment

One ml from the rinsed water was taken and transferred into a universal Bijou bottles containing 9 ml of selenite F broth aseptically and incubated at 45^0C for 24 hours in the incubator.

Culturing

A loopful from the enrichment flask was then taken and streaked on the Salmonella-Shigella Agar. From the homogenate 0.25ml was taken and then serially diluted sample was plated on Salmonella-Shigella Agar.

Incubation

All plates were incubated at 37^{o}C for 24 hours in the incubator. After 24 hours of incubation the plates were removed from the incubator and observed for the characteristic colonies. Subcultures of the characteristic colonies were made to obtained pure cultures.

Gram staining and Microscopy

From each plate that showed growth, smears of the colonies were made on glass slides. This was carried out by placing a drop of normal saline on the glass slide using a sterile wire loop, the colony was then picked and emulsified into the normal saline and spread out to make a thin layer using a sterile wire loop. The smear was allowed to air dry and was then heat fixed by passing the slides on a bunsen flame three times. The slides were then placed on a clean rack and flooded with crystal violet (a primary dye) and allowed to stand for 60secs, the slides were rinsed and Grams iodine (a mordant) was poured on the slides and allowed to stand for another 60secs. The slides were rinsed again and then flooded with acetone for 10secs and washed off immediately. The slides were then flooded with safranine for counter staining for 1minutes and washed off. The slides were blotted dry and a drop of oil immersion was placed on each smear. The slides were viewed under the microscope using the x 100 magnification. The gram reaction and shape of the microorganisms were observed and noted.

BIOCHEMICAL TESTS

Sugar fermentation in Kligler Iron Agar (KIA)

A sterilized straight wire needle was used to pick the pure culture and then it was strreaked on the surface of the slant and was stabbed down to the butt. The tubes were capped loosely using cotton wool and incubated at 37^{o}C for 24 hours.

Citrate utilization test

Bijou bottles containing Simmons Citrate Agar slants were inoculated and incubated at 37^{o}C for 24-72 hours. The development of a deep blue colour indicates a positive result.

Motility test

The motility medium was inoculated by making a line stab with a straight wire needle to a depth of 1-2cm short of the bottom of the tube. The tubes were incubated at 35^{o}C for 24 - 48 hours. At the end of the incubation period the tubes were examined. The line of inoculation could not be sharply defined and the rest of the medium would be somewhat cloudy if the organism is motile. If the organism is not moving growth would be restricted to the line of the inoculation which becomes sharply defined while the rest of the medium remains clear.

Methyl Red and VogesProskauer test

Five ml of MR-VP broth was inoculated and incubated for 48-72 hours at 35^{o}C. After the period of incubation, 1ml of the broth was transferred to a small serological tube 2-3 drops of methyl red was added to this small quantity. A red colour on the addition of the indicator signifies a positive methyl red test. A yellow colour signifies a negative test. To the rest of the broth in the original tube, 5 drops of 40% potassium hydroxide (KOH) was added followed by 15 drops of 5% naphthol in ethanol. It was then shook loosen the cap of the tube and then placed in a

sloping position. The development of a red colour starting from the liquid-air interface within an hour indicates a VP positive test. No colour change occurs in a VP negative test.

Use of vinegar solution and exposure time on *Salmonella*

Different concentrations of 0.5, 1.5 and 2.5% of vinegar were used to wash 10g of each vegetable (carrot, lettuce and cabbage) as described by Amoah *et al.*, 2007b. One ml of the solution was transferred into an enrichment media (selenite F broth).

It was then incubated at 47^{o}C for 24 hours. At different exposure time (0-10 mins).From the enrichment flask 0.1ml was then taken and streaked on Salmonella-Shigella Agar and the plates were incubated at 37^{o}C for 24 hours. The plates were observed for *Salmonella* growth at the end of the incubation time. Colonies were counted and recorded.

Use of salt solutions and exposure time on *Salmonella*

Five, 10 and 15% of salt concentrations were used to wash 10g of each vegetable (Amoah *et al.*, 2007b). One ml of the rinsed solution was transferred into an enrichment media (selenite F broth) at different exposure time (0-10mins). It was then incubated at 47^{o}C for 24 hours. The Salmonella-Shigella Agar medium was streaked using 0.1ml of the rinsed water and the plates were incubated at 37^{o}C for 24 hours. From the plates showed growth. Colonies were counted and recorded.

Use of potash solutions and exposure time on *Salmonella* isolates

Five, 10 and 15% of potash concentrations were used to wash 10g of each vegetable (Amoah *et al*., 2007b). One ml of the rinsed solution was transferred into an enrichment media (selenite F broth) at different exposure time (0-10mins). It was then incubated at 47^{o}C for 24hours. The Salmonella-Shigella Agar medium was streaked using 0.1ml of the rinsed water and the plates were incubated at 37^{o}C for 24hours. From the plates showed growth. Colonies were counted and recorded.

RESULTS

Cultural Characteristic, Number and Percentage of Presumptive *Salmonella species* Obtained from Rinsed Water

The table below shows the colonial morphology, number and percentage of presumptive *Salmonella species* in the three different vegetable samples collected.

Table 1: **Colonial morphology, number and percentage of presumptive *Salmonella species* in vegetable samples**

Colonial morphology	Number and % of Samples Inference			Inference
	Carrots	Lettuce	Cabbage	
Non lactose fermenting	5 out of 10	5 out of 10	6 out of 10	Confirmed
Colonies with heavy H_2S production	-50%	-50%	-50%	*Salmonella*

Biochemical Characterization

Table 2 below shows the biochemical characterization of *Salmonella species* obtained from Carrots.

Out of the 5 isolates obtained from carrots; 4 isolates were *Salmonella typhi*, while only an isolate was unidentified *Salmonella species*. *Salmonella typhi* and unidentified *Salmonella species* were differentiated by the citrate utilization test.

Table 2: Biochemical characteristics of *Salmonella species* obtained from Carrots

Sample Number Isolate	KIA	Motility	Citrate	MR	VP
S. typhi					
1	K/A^+ H_2S	+	+	+	-
2	K/A^+ H_2S	++	+	-	*S. typhi*
H_2S	H_2S				
3	K/A^+	+++	-	*S. typhi*	
S. typhi					
4	K/A^+	+	+	+	-
S. Specie	H_2S				
5	K/A^+ H_2S	+	-	+	-

Key:

K	=	Alkaline
A	=	Acid
H_2S	=	Hydrogen Sulphide
-	=	Negative
+	=	Positive
KIA	=	Kligler Iron Agar test
Motility	=	Motility test
Citrate	=	Citrate utilization test
MR	=	Methyl Red test
VP	=	Voges – Proskauer test

Table 3 shows the biochemical characteristics of *Salmonella* isolate obtained from lettuce.

Out of 5 isolates obtained from lettuce; 3 isolate were specifically *Salmonella typhi* while the other 2 were unidentified *Salmonella species*. The former were differentiated from the latter by the citrate utilization test.

Table 3: Biochemical characteristics of *Salmonella species* obtained from Lettuces

Sample number Isolate	KIA	Motility	Citrate	MR	VP
1 *S.typhi*	K/A^+	+	+	+	-
2 H_2S	K/A^+ H_2S	+ +	+	-	*S. typhi*
3 H_2S	K/A^+	+++	-	*S.typhi*	
4 *S.species*	K/A^+ H_2S	+	-	+	-
5 *S.specie*	K/A^+ H_2S	+	-	+	-

Table 4 shows the biochemical characteristics of *Salmonella* isolate obtained from Cabbage.

Out of 5 isolates obtained from Cabbage; 2 isolate were specifically *Salmonella typhi* while the other 3 were unidentified *Salmonella species*. The former were differentiated from the latter by the citrate utilization test.

Table 4: Biochemical characteristics of *Salmonella species* obtained from Cabbages

Sample number Isolate	KIA	Motility	Citrate	MR	VP
1 *S.typhi*	K/A^+	+	+	+	-
2	H_2S H_2S	+ +	+	-	*S. typhi*
3 *S.species*	K/A^+	+	-	+	-
4 H_2S	K/A^+ H_2S	+	-	+	-
5 *S.specie*	K/A^+ H_2S	+	-	+	-

Table 5: **Effect of Sanitizing agents at different concentrations and different exposure time (0-10min) on *Salmonella species* in rinsed water of vegetables sampled in Samaru Market**

Conc. of sanitizing agents (%)	Exposure time (Minutes) of *Salmonella species* counts *(cfu/ml)*		
	0	5	10
Vinegar			
0.5	TNTC	7.5×10^4	7.0×10^4
1.5	TNTC	7.2×10^4	No growth
2.5	TNTC	4.0×10^4	No growth
Salt			
5	TNTC	TNTC	TNTC
10	TNTC	8.5×10^4	8.4×10^4
15	TNTC	7.9×10^4	8.0×10^4
Potash			
5	TNTC	TNTC	TNTC
10	TNTC	TNTC	8.6×10^4
15	TNTC	8.8×10^4	8.5×10^4

Key: TNCT = Too Numerous To Count.

Table 5 shows different concentrations of different sanitizing agents (vinegar, salt and potash) at different exposure time on the *Salmonella* isolates.

DISCUSSION

The results of this study showed that, the vegetables (Carrots, Cabbage and Lettuce) sampled in Samaru market are contaminated with *Salmonella species* which is in accordance with Amoah *et al*., 2011 ,that also shows that; market offer some options to support either active (e.g through washing with clean water) or passive decontamination (e.g supporting die-off through appropriate storage) as well as possibilities to prevent additional contamination (through improved display or hygienic handling).Presence of *Salmonella species* in these vegetable salad samples is a direct reflection of sanitary quality of the cultivation water, harvesting, transportation, storage and processing of the produce (Beuchat, 1996).

The result also showed that as the concentration of vinegar increases the number of colonies formed by *Salmonella* decreases with increasing exposure time. Salt shows a slower reduction in the number of colonies formed compared to vinegar while potash shows the slowest reduction in all the three different vegetable salad samples observed. According to Parish *et al.* (2003) the efficacy of the method used to reduce microbial population usually depends on the type of treatment, type and physiology of the target microorganisms, characteristics of produce surface, exposure time and concentration of sanitizer, pH and temperature. This observation is in agreement with the observation made by Amoah *et al*. (2007a). This could be due to the

concentration of salts solution used in washing the vegetables since it was prepared to taste of the food vendor. Higher salt concentration of 23 and 35 mg/L had a considerable deteriorating effect on lettuce leaves (Amoah *et al*., 2007a). The low concentration of the sanitizer might account for low reducing power of the salt solutions compared to vinegar. Because vinegar is expensive therefore not easily affordable, the potential of adoption by street kitchen vendors is low.

CONCLUSION

This study showed that the vegetables (Cabbage, Carrot and Lettuce) sold in Samaru marke are highly contaminated with enteric pathogens such as *Salmonella species* and the contamination is above safe recommendation level.

It can be concluded that vinegar is the most potent or effective sanitizer than common salt (NaCl) while potash has little or no significant effect on microbial load reduction (*Salmonella*).

RECOMMENDATIONS

Ensuring food safety to protect public health and promote economic development remains a significant challenge in developing countries where untreated waste water and manure fertilizer are used in vegetable farming. It is recommended that:-

- Vegetable farmers using waste water or untreated water and animal dung (night soils) as fertilizer should be advised to use potable water for irrigation and compost as fertilizer.

- Vegetable sellers in the market should be educated on improved post-harvest handling practices and encourage adoption to reduce contamination.

- Street kitchen vendors should be educated and encouraged to use improved washing methods during food preparation like the use of vinegar.

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