



THE EFFECT OF SALT CONCENTRATIONS ON BACTERIAL POPULATION OF SOME FRESH VEGETABLES

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ABSTRACT

Green vegetables have great nutritional and therapeutic values and are usually used as the main ingredients for preparing fresh vegetable/salad for consumption. The aim of this work is to determine the bacterial population in different fresh vegetables and to adopt the most appropriate method for washing to reduce the bacterial population prior to consumption using salt solutions. The samples experiments were made of 4 levels of treatments (5%, 7% and 8% salt solutions and sterile distilled water as control) and the effectiveness of the treatments in reducing the bacteria population determined. The untreated vegetables had total average aerobic plate counts of 7.83×10^5 – 3.55×10^6 CFU/g, and coliforms ranges of 377 – >1100 MPN/g. However, samples treated with 5%, 7% and 8% salt concentrations had aerobic plate counts of 1.55×10^5 – 1.07×10^6 CFU/g, 1.21×10^4 – 2.28×10^4 CFU/g and 3.96×10^3 – 9.86×10^3 cfu/g respectively; whereas their coliforms ranges are respectively 13 – >1100 MPN/g, <3 – 82 MPN/g and <3 – 48 MPN/g.

INTRODUCTION

Lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*) and parsley (*Petroselinum crispum*) were selected as the study samples because they are among the commonest vegetables used by the surrounding communities and mostly consumed fresh or added to some cooked foods like rice. Lettuce (*Lactuca sativa*), which includes loose leaf and heading types, is a member of the Asteraceae family. “Leafy greens” or “greens” are broad terms used for a number of vegetable crops with edible leaves. It is an

annual plant native to the Mediterranean area. Lettuce contains useful amounts of several nutrients including vitamins A and C; and minerals calcium and iron. The nutrient content is highest in the darker green, outer leaves. Each head contains 65 to 70 kilocalories. Cabbage (*Brassica oleracea*) is a cool season crop with a high cold tolerance; however, heads may bolt (flower prematurely) in warm temperatures. Cabbage is suitable for both fresh and processed products (James, 2000).

Parsley is a typical root vegetable. Parsley is an herb whose leaves are used either as a garnish or as a spice for flavoring in food. It belongs to the same family as the carrot. Parsley is a bright green biennial that seeds during the second year (Pokluda, 2003).

Contaminations in food and agricultural products may occur in every stage of the food supply chain, from the field to the table, i.e. production, harvesting, processing, storage and distribution, calling for proper decontamination and ensuring food safety at each of these stages using an effective antimicrobial agent.

Water activity value of saturated salt solution is only about 0.75 and a number of microorganism varieties are able to grow even below this limit, it is impossible to protect food stuff reliably from all microbial attack by using salt alone, unless the flavor becomes completely unacceptable. The resulting osmotic removal of water from the food reduces the water activity to a level according to the quantity of salt added. In addition, bacteria are more inclined to accumulate certain amino acids when the water activity is lowered, thus salt inhibits their growth (Lee, 2004).

This investigation was undertaken to assess the effectiveness of various concentrations of salt in the reduction of bacterial load of fresh lettuce which is a major food component nowadays and mostly eaten raw.

The mesophilic microorganisms are among the more general and extensively microbiological indicators of food quality, indicating the adequacy of temperature and sanitation control during processing, transport, and storage, and revealing sources of contamination (John and Alicia, 2001).

MATERIALS AND METHODS

Sample Collection

Among the vegetables sold or available in the market, lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*) and parsley (*Petroselinum crispum*) were selected as the experimental materials.

Samples were purchased for the analysis from one market only (Wambai market, Kano State) throughout the period of the analysis. Sampling visits were made prior to analysis. On each sampling visit, only one sample was collected in a sterile polythene bag immediately transported to the laboratory (Adams and Moss, 1999).

Preparation of salt concentrations

Three different concentrations of salt solution were prepared in the analysis (i.e. 5%, 7% and 8% gram/volume). According to the procedure adopted by Cheesbrough (2010), 5g, 7g and 8g of salt crystals were each dissolved in 100ml of the sterilized solvent (buffered peptone water) to make 5%, 7% and 8% g/v concentrations before mixing with the sample.

Aerobic Mesophilic Count

For cabbage, fresh leaves taken from the outer, middle and inner sections of the samples were analyzed for aerobic mesophilic organisms, and coliforms (Atter *et al.*, 2014).

Sample Preparation and Serial Dilution

According to method described by Nwachukwu and Chukwu, (2013), twenty-five grams (25 g) of each sample was aseptically collected and placed in a sterile blender to which 225 ml of buffered peptone water was added and homogenized for 2 min at normal speed. For the vegetables treated with salt, the vegetable sample in each of the solutions was allowed to stand for 10 min and thereafter homogenized and 1ml of the homogenate was 10 fold serially diluted.

Serial dilution was carried out according to procedure described by Madigan *et al.* (2012) as follows:

Using sterile pipette, 1ml of sample was withdrawn and mixed with 9ml of diluent (buffered peptone water) contained in a test tube to make 10-fold (10^{-1}) dilution. The dilution was well shaken.

From the 10-fold (10^{-1}) dilution, 1ml of the dilution was withdrawn and transferred to 9ml of the diluent to obtain 100-fold (10^{-2}) dilution.

Successive dilutions of the sample (10^{-3} , 10^{-4} and 10^{-5}) were made following the above procedure.

Plating of Sample

According to Madigan *et al.* (2012), pour-plate method was used as follows:

One ml of serially diluted sample was pipetted into a sterile Petri plate.

Melted agar medium (melted nutrient agar), tempered to just about gelling temperature (about 45°C), was then added and mixed well by gently swirling the plate on the bench top.

The molten nutrient agar was allowed to solidify.

The plates were then incubated at 37°C for 24 hours.

Colonies on a plate (between 30-300) were counted and recorded

Coliform Count

This was carried out according to the method described by Atlas, (1997). A set up consisting of nine (9) test tubes each containing 9ml of lactose broth and inverted Durham tube, were autoclaved to expel air and to sterilize. Inoculation was

made from the serially diluted samples as follows:

From the 1:10 dilution, 1ml of inoculum was transferred to each of the first three of the 9 test tubes containing 9ml of lactose broth;

Then 1ml also was transferred from 1:100 dilution to each of the second set of three test tubes of lactose broth; and

Finally 1ml of inoculum was transferred from 1:1000 dilution to each of the last set of three test tubes.

All the 9 test tubes were incubated at 37°C for 24 hours and another 24 hours in the absence of gas. Following 24 or 48 hours of incubation the tubes were observed for gas production and the number of gas positive tubes was compared with the most probable number (MPN) table to estimate the most probable number of coliforms per gram of the sample.

RESULTS

The results of the bacteria loads from the various samples were presented in Table 1 and Table 2. The samples analyzed include cabbage, lettuce, and parsley. The untreated vegetables had total average aerobic plate counts between 7.83×10^5 to 3.55×10^6 CFU/g, and coliform count ranging between 377 and >1100 MPN/g. However, samples treated with 5%, 7% and 8% salt concentrations had aerobic plate counts of 1.55×10^5 - 1.07×10^6 cfu/g, 1.21×10^4 - 2.28×10^4 CFU/g and 3.96×10^3 - 9.86×10^3 CFU/g respectively; whereas their coliforms ranges are respectively 13 - >1100 MPN/g, <3 - 82 MPN/g and <3 - 48 MPN/g.

Table 1: Effect of salt concentrations on the aerobic mesophilic bacterial counts of cabbage, lettuce and parsley

Sample	Concentration of salt (% g/v)	Total Average APC (CFU/g)
Cabbage	0.00	7.83×10^5
	5.00	3.25×10^5
	7.00	2.22×10^4
	8.00	8.08×10^3
Lettuce	0.00	3.55×10^6
	5.00	1.07×10^6
	7.00	2.28×10^4
	8.00	9.86×10^3
parsley	0.00	3.38×10^5
	5.00	1.55×10^5
	7.00	1.21×10^4
	8.00	3.96×10^3

Key:

APC= Aerobic plate count

Table 2: Effect of salt concentrations on coliform counts of cabbage, lettuce and parsley

Sample	Concentration of salt (% g/v)	Average MPN/g of sample
Cabbage	0.00	421
	5.00	394
	7.00	15
	8.00	06
Lettuce	0.00	>2400
	5.00	>2400
	7.00	82
	8.00	48
parsley	0.00	377
	5.00	13
	7.00	<3
	8.00	<3

Key:

MPN – Most Probable Number

Table 3: Comparative effects of different salt concentrations on bacterial population of vegetable samples

Salt concentrations(g/v)	Aerobic plate count (CFU/g)			Coliform count (MPN/g)		
	cabbage	Lettuce	Parsley	cabbage	Lettuce	parsley
0.00	7.83×10^5	3.55×10^6	3.38×10^5	421	>2400	377
5.00	3.25×10^5	1.07×10^6	1.55×10^5	394	>2400	13
7.00	2.22×10^4	2.28×10^4	1.21×10^4	15	82	<3
8.00	8.08×10^3	9.86×10^3	3.96×10^3	06	48	<3

Key:

CFU – Colony Forming Units

MPN – Most Probable Number

DISCUSSION

The vegetables used as experimental material in this study are among the commonest vegetables used by the surrounding communities and mostly eaten fresh or added to some cooked foods like rice.

The results showed that generally the untreated fresh green vegetables (cabbage, lettuce and parsley leaves) carried the highest bacteria population however the mean bacterial population decreased with increase in salt concentrations. The aerobic mesophilic counts of 7.83×10^5 , 3.55×10^6 and 3.38×10^5 for untreated cabbage, lettuce and parsley samples respectively while 3.25×10^5 , 1.07×10^6 and $1.55 \times 10^5 - 10^6$ CFU/g for cabbage, lettuce and parsley samples treated with 5% salt solution, 2.22×10^4 , 2.28×10^4 and 1.21×10^4 or cabbage, lettuce and parsley samples treated with 7% salt solution while 8.08×10^3 , 9.89×10^3 and 3.96×10^3 for cabbage, lettuce and parsley samples treated with 8% salt solutions respectively. Similar trends were recorded for the coliform population: untreated vegetables had the highest coliform bacteria that ranged from 377 - >1100MPN/g, whereas the highest concentration of salt used recorded the lowest counts ranging from <3 - 48MPN/g. From the study it was observed that the treatment with 8% salt solution was most effective in reducing the bacteria population on the samples analyzed followed by 7% salt solution and 5% salt solution, the least effective. This implies that inhibition of bacteria increases with increase in salt concentrations.

The high bacteria population obtained for the untreated fresh vegetables may be attributed to a number of factors. Since there is the need for the fertilization of the soil most of farmers apply poultry litter without adequate composting and this

constitutes a potential source of food contamination especially leafy vegetables (Drechsel *et al.*, 2000). The poor handling and hygienic practices by both the farmers and sellers of vegetable may makes it prone to contamination. Apart from handlers of food, environmental surfaces and equipments can also be sources of contamination, especially with *Staphylococcus aureus* (Atter *et al.*, 2014). There was no significant difference between the mean populations before and after treatment with 5% sodium chloride solution for the total mesophilic aerobic bacteria. The treatments with 7% and 8% sodium chloride however yielded significant reduction of the mesophilic aerobic bacteria and coliform counts, which is in agreement with the work of Ana *et al.*, (2012) which showed that the disinfection of lettuce with salt solutions reduced the counts by $10^1 - 10^2$ CFU/g (Table 1). Salting significantly reduce initial microbial loads but result in enhanced survival and/or growth during storage (Maria *et al.*, 2009). In fact, the main effect of sanitizing treatments for fresh-cut produce is to reduce their microbial load and keep them from contamination rather than having a preservative effect (Maria *et al.*, 2009). Since the water activity value of saturated salt solution is only about 0.75 and a number of microorganism varieties are able to grow even below this limit, it is impossible to protect food stuff reliably from all microbial attack by using salt alone, unless the flavor becomes completely unacceptable. The resulting osmotic removal of water from the food reduces the water activity to a level according to the quantity of salt added. In addition, bacteria are more inclined to accumulate certain amino acids when the water activity is lowered, thus salt inhibits their growth (Lee, 2004).

CONCLUSION

The results of this study indicated that there is reduction in the aerobic mesophilic bacteria and coliform counts of vegetables treated with salt solutions. Treatment with 7% and 8% salt solutions substantially reduce the bacterial population with 8% concentration being most effective. Different concentrations of sodium chloride are therefore effective and even more effective with increase in concentrations in reducing the microbial contents of vegetables.

RECOMMENDATIONS

The following recommendations are made based on the study carried out:

- (1) Vegetable growers should be educated on the hazards associated

with using polluted or sewage irrigation water for irrigating vegetables in general on the consumer. If possible vegetable farmer's access to clean and safe water should be given more attention by the appropriate authorities.

- (2) There is the need for public education or awareness creation on sanitizing vegetables with salt solution prior to consumption
- (3) Better sanitizing chemicals such as chlorine, ozone, ionized water, etc. should be used to reduce the microbial contents of fresh vegetables.
- (4) Vegetables treated with salt solutions should be rewashed with clean water to reduce the salt burden.

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