

## Incidence and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on salad vegetables

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### Abstract

Salad vegetables contaminated with pathogens can cause food poisoning. The isolation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* from different salad vegetables; cucumber, cabbage, carrot, and lettuce was carried out. *E. coli* O157:H7 was isolated from all the salad vegetables while *L. monocytogenes* was isolated from only cabbage and lettuce. The effect of different storage temperatures on the survival and growth of *E. coli* O157:H7 and *L. monocytogenes* on cabbage and lettuce was determined. Known population of each isolate was inoculated into sliced cabbage and lettuce separately and stored at 5°C (refrigerator temperature) and 28°C (room temperature) for 10 days. Bacteria were counted on daily basis. Result showed initial increase in most cases on second and/or third day followed by decrease in bacterial population all through storage. There was no growth towards the end of storage at 28°C. In all cases there was decrease in pH. The count of *E. coli* O157:H7 ranged from  $3.6 \times 10^3 - 4.0 \times 10^2$  cfu/g in salad vegetables stored at 5°C and the pH, 7.11 - 5.66 while the count at 28°C was  $3.9 \times 10^3 - 1.0 \times 10^2$  cfu/g with pH 7.11 - 4.06. *L. monocytogenes* count in salad vegetables stored at 5°C was  $1.51 \times 10^4 - 4.8 \times 10^2$  cfu/g and pH was 7.12 - 5.84. At 28°C, the count was  $1.49 \times 10^4 - 2.0 \times 10^2$  cfu/g and pH was 7.14 - 4.14. Both bacterial pathogens survived at storage temperatures at which salad vegetables are normally stored in practice. Their presence and survival in salad vegetables call for public health concern because salad vegetables receive little or no heat treatment before consumption.

### Keywords

*Escherichia coli* O157:H7  
*Listeria monocytogenes*  
Salad vegetables

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### Introduction

Salad vegetables are consumed with little or no heat treatment and sometimes without washing and peeling. Consumers are therefore exposed to the risk of food borne diseases. Vegetables can become contaminated with pathogenic microorganisms from harvesting equipments, transport containers, and domestic animals. The pathogenic microorganisms which reside in intestinal tracts of animals or humans are more likely to contaminate vegetables through faeces, sewage, untreated irrigation water or surface water (Harris *et al.*, 2003). 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 contamination (Amoah *et al.*, 2009). Unsafe water used for rinsing the vegetables and sprinkling to keep them fresh is also a source of contamination (Mensah *et al.*, 2002). Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetables and fruits. Use of manure has led to concern for the potential of contamination of minimally processed vegetables such as salad vegetables (e.g. lettuce, cabbage, cucumber and carrot) with

enteric pathogens (Ingham *et al.*, 2004). There is a great variation in the number of microorganisms on vegetables, and factors like nutritional substances, microbial competition, structural damages on plant (wounds) and the potential in internalization of pathogens, affect the proliferation of pathogens on vegetables. However, when infective dose of pathogens is low, the persistence of microorganisms is as important as proliferation of these pathogens (Aruscavage *et al.*, 2006). Khandaghi *et al.* (2010) examined farmlands in their study, *E. coli* O157:H7 was found to be able to persist for a long time in the soil and could contaminate crops such as raw vegetables. Long term persistence of this organism that poses a risk of transmission of pathogens both by direct contact or ingestion of produce grown in contaminated farmlands has also been demonstrated previously (Avery *et al.*, 2005; Fremaux *et al.*, 2007; Fukushima *et al.*, 1999; Lau and Ingham, 2001). Many authors have isolated microorganisms from vegetable salad. Uzeh *et al.* (2009) isolated bacteria and molds from pre-packed mixed vegetable salad and salad vegetables from some retail outlets in Lagos, Nigeria. Little *et al.* (2007) reported that *L. monocytogenes* had been detected in ready-to-eat mixed salads in the

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The objectives of this research therefore are to: 1. Isolate, characterize and identify *E. coli* O157:H7 and *L. monocytogenes* from different salad vegetables. 2. Study the effect of temperature on the survival and growth of *E. coli* O157:H7 and *L. monocytogenes* on cabbage and lettuce and 3. Determine the changes in pH during storage.

## Materials and Methods

### Collection of salad vegetables

Different salad vegetables; carrots, cucumber, lettuce and cabbage were collected from retail stores at Yaba market, Lagos, Nigeria. Samples were collected in sterile containers and taken to the laboratory for analysis without delay.

### Determination of pH

Standardized pH meter was used to record pH of samples during storage.

### Isolation of *Escherichia coli* O157:H7

From each salad vegetable 25 g was weighed and shaken vigorously in 225 ml of sterile distilled water and serial dilutions were made. Aliquot of 0.1 ml of each dilution was inoculated onto MacConkey agar plates in duplicate using the spread plate method. All the plates were incubated at 37°C for 24 hr. Discrete colonies that appeared pink in colour were subcultured to obtain pure culture. Sorbitol MacConkey agar was inoculated with colonies from the pure culture and incubated at 37°C for 24 hr. Colonies that appeared colourless were subjected to further tests.

### Isolation of *Listeria monocytogenes*

From each salad vegetable 25 g was weighed and shaken vigorously in 225 ml of *Listeria* enrichment broth base (UVM formulation) and incubated for 24 hr at 37°C. A loopful of the broth culture was used to inoculate Palcam agar base and incubated for 24 hr at 37°C. Colonies that developed were sub cultured to obtain pure culture which was subjected to further tests for confirmation of *Listeria monocytogenes*.

### Characterisation and identification of isolates

Pure colonies of *Escherichia coli* O157:H7 and *Listeria monocytogenes* were identified on the basis of their cultural, morphological and biochemical characteristics. The biochemical tests performed include catalase, indole, oxidase, urease activity, methyl red, citrate utilization, acid and gas production from sugars.

### Cultural characteristics

The shape, size, pigmentation, elevation, consistency and marginal characteristics were examined on Sorbitol MacConkey agar and Palcam agar base after incubation.

### Motility test

This was performed using the agar stab inoculation method. A straight sterile inoculating needle was used to pick the test organism which was used to inoculate the agar medium by stabbing straight down into the medium. For *E. coli* O157:H7, the stabbed medium was incubated for 24 hr at 37°C while for *L. monocytogenes*, it was incubated for 48 hr at 22°C. Growth of the organism away from the line of inoculation showed motility.

### Effect of temperature on the survival and growth of *E. coli* O157:H7 and *L. monocytogenes* on salad vegetables

Broth culture of *E. coli* O157:H7 of a large population suspension of  $10^6$ - $10^8$  cfu/ml was prepared. From this, 50 ml was used to inoculate 1 kg each of chopped cabbage and lettuce. From each, 200 g was taken in triplicate and incubated at 5°C, 28°C and 37°C for 10 days. A 25 g sample of each vegetable was taken before incubation in order to perform an initial count of the organism. At daily interval 25 g of sample was taken and added to 225 ml of 0.1% sterile peptone water in a sterile conical flask and shaken thoroughly. The fluid was serially diluted and plated in duplicate on Sorbitol MacConkey agar plates which were incubated at 37°C for 24 hr and colonies that developed were counted. The procedure above was repeated for *Listeria monocytogenes* except that plating was done on Palcam agar base and incubated at 37°C for 24hr at the end of which colonies that developed were also counted.

## Result

*E. coli* O157:H7 was isolated from all the salad vegetables. *L. monocytogenes* was isolated from cabbage and lettuce. It was however not isolated from carrot and cucumber. In most cases, there was initial increase in bacterial population on second and/or third day followed by decrease all through storage (Figures 1 and 2). There was no growth towards the end of storage at 28°C (Figures 1 and 2). In all cases there was decrease in pH (Figures 3 and 4). On cabbage, the count of *E. coli* O157:H7 ranged from  $2.7 \times 10^3$  -  $4.0 \times 10^2$  cfu/g and the pH, 7.09 - 5.72 at 5°C, while at 28°C the count was  $2.5 \times 10^3$  -  $1.0 \times 10^2$  cfu/g and the pH, 7.09 - 4.15 (Figures 1 and 3).

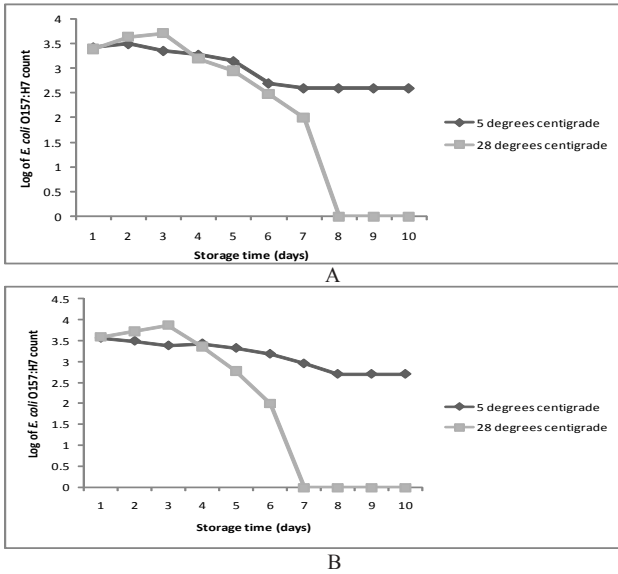


Figure 1. Survival of *Escherichia coli* O157:H7 on cabbage (A) and lettuce (B) stored at different temperatures

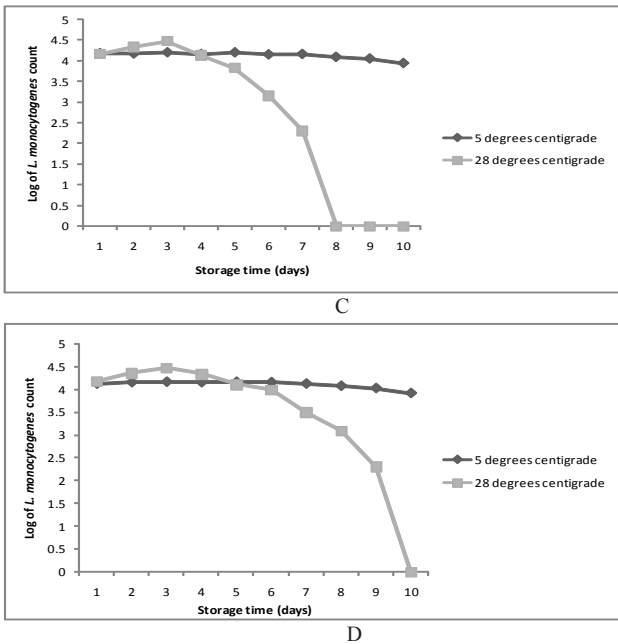


Figure 2. Survival of *Listeria monocytogenes* on cabbage (C) and lettuce (D) stored at different temperatures

The count of *E. coli* O157:H7 on lettuce at 5°C was in the range of  $3.6 \times 10^3$  -  $5.0 \times 10^2$  cfu/g and the pH decreased from 7.11 to 5.66. At 28°C, the count was  $3.9 \times 10^3$  -  $1.0 \times 10^2$  cfu/g and the pH decreased from 7.11 to 4.06 (Fig 1 & 3). For *L. monocytogenes* on cabbage, the count ranged from  $1.51 \times 10^4$  -  $4.8 \times 10^2$  cfu/g and the pH, 7.12 - 5.92 at 5°C. The count at 28°C was  $1.43 \times 10^4$  -  $2.0 \times 10^2$  cfu/g, while pH was 7.12 - 4.14 (Figures 2 and 4). In the case of lettuce, the count of *L. monocytogenes* at 5°C ranged from  $1.32 \times 10^4$  -  $3.9 \times 10^3$  cfu/g and the pH decreased from 7.12 to 5.84, while at 28°C, the count was  $1.49 \times 10^4$  -  $2.0 \times 10^2$  cfu/g and pH was 7.14 - 4.34 (Figures 2

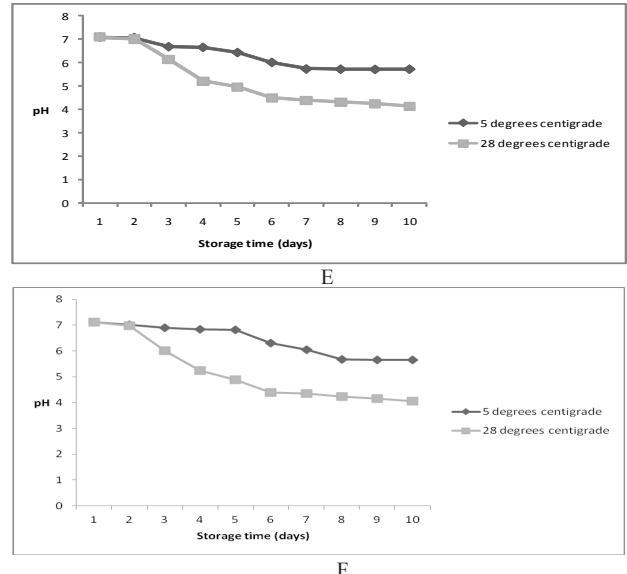


Figure 3. Changes in pH during survival of *Escherichia coli* O157:H7 on cabbage (E) and lettuce (F) stored at different temperatures

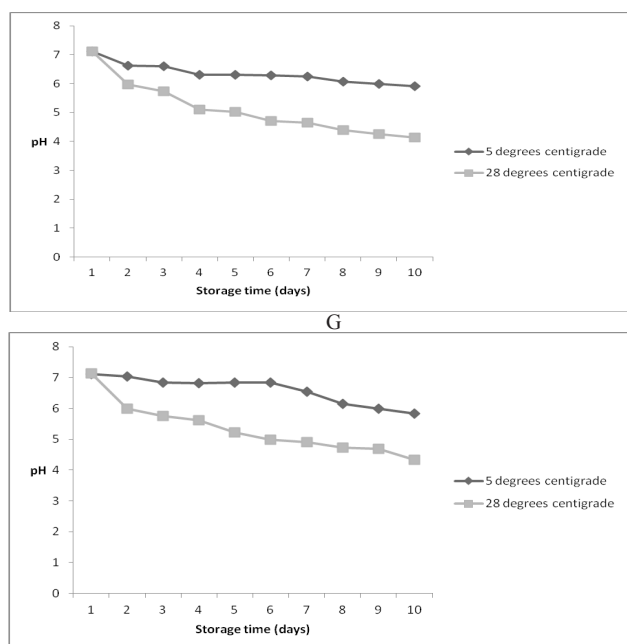


Figure 4. Changes in pH during survival of *Listeria monocytogenes* on cabbage (G) and lettuce (H) stored at different temperatures

and 4).

**Discussion**

From this study *Escherichia coli* O157:H7 was isolated from all the salad vegetables. The source of this organism may be from human faeces, cow dung or chicken droppings which farmers of this fresh produce usually use as manure in Nigeria. It is very likely that humans, cow, and chicken can share this pathogen in their faeces. These sources of manure are not usually treated to remove pathogens before

application to the soil in the farm. It could also be from contaminated water, soil or handling of the salad vegetables in agreement with Johannessen *et al.* (2002). However, *L. monocytogenes* was not isolated from carrot and cucumber but was isolated from other salad vegetables. It has been established that raw carrot juice is lethal to *L. monocytogenes* (Beuchat and Brackett, 1990). 6-methoxymellein, a known carrot phytoalexin, inhibits the growth of several fungi and bacteria (Kurosaki and Nishi, 1983). After an initial increase, there was decrease in the population of *E. coli* O157:H7 and *L. monocytogenes* on stored salad vegetables. This may be due to effect of decrease in pH of the vegetables during storage and competition with other microorganisms present. Similar results were obtained by Arias *et al.* (2001). The decrease in pH may be as a result of possible fermentative capability of the pathogens or presence of lactic acid bacteria which have increased the acidity of the stored vegetables also by fermentation. Viable populations of *E. coli* O157:H7 and *L. monocytogenes* remained at the end of storage of vegetables at 5°C with similar report by Francis *et al.* (2001). However there was no viable population at the end of storage at 28°C. This may be the effect of reduced pH to less than 4.40.

The high association of *E. coli* O157:H7 and *L. monocytogenes* with these salad vegetables coupled with their survival on vegetables irrespective of the decrease in their population during storage, creates a potential health concern. This concern is heightened by the fact that these vegetables are used in combination to make green salads which are consumed raw. Secondly they are infective at very low dose which can be as low as 10 cells of *E. coli* O157:H7 and 100 to 1000 cells of *L. Monocytogenes* in a portion of food for immuno-compromised people (FDA, 1993). Therefore, in order to minimize the risk of infectious disease, it is advisable to use safe source of manure and follow better hygienic practices in irrigation, harvesting and handling of these crops.

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