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Time course study on the growth of *Salmonella* Enteritidis on raw vegetables used in sandwiches

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Abstract: A time course study on the growth of *Salmonella* Enteritidis on raw vegetables used a part of the ingredients for sandwiches at room temperature and at 4°C, with initial microbial load of log 3 and log 1 spiked onto the lettuce and cucumber slices. The growth of *Salmonella* Enteritidis were higher at room temperature and were dose dependent. The information obtained in this study may contribute towards food handling solutions in order to be able to control the food safety of fresh produce.

Keywords: *Salmonella* Enteritidis, time, temperature, raw vegetable

Introduction

The market sales of fresh cut vegetables have expanded tremendously in recent decades as a result of changes in consumer attitude toward fresh cut vegetables, especially consumption of fresh cut such as lettuce and carrot used in salad preparation (Giovannaci *et al*., 2008). Garcia *et al*. (1987) reported that natural contaminants of fresh produce and fresh cut vegetables may include bacteria, yeast and mould. Storage of the fresh cut fruits and vegetables at relatively high CO_2 concentrations of 5 to 10% along with low temperature conditions may result in the inhibitions of gram negative bacteria such as *Pseudomonas* spp., or Enterobacteriace. This gram negative bacteria are considered as common spoilage bacteria in fresh cut vegetables due to their higher growth rate at lower temperature (Jacxsens *et al*., 1999).

Fresh produce is now recognized as emerging vehicle causing food borne illnesses, demonstrating a shift from traditional problems with foods from animal origin to fresh foods such as produce (VWA-EFSA, 2008). The increased concern about the microbiological contamination of fresh produce and its potential contribution and relation to food borne illness has been indicated by several surveillance studies in our local setting (Noorzaleha *et al*., 2003; Chai *et al*., 2007; Jeyaletchumi *et al*., 2010a, 2010b; Tunung *et al*., 2011). Therefore, the objective of this study is to analyse the growth of *Salmonella* Enteritidis artificially spiked on cucumber and lettuce used as part of the ingredients in egg sandwiches to provide insights into their capabilities to be transferred via fresh produce.

Materials and Methods

Bacterial cultures

The *S.* Enteritidis strain used in this study was from the culture collection of the Food Safety Laboratory, Universiti Putra Malaysia and the working culture was maintained on Tryptone soya agar slants (TSA) at 4°C.

Inoculum preparation

S. Enteritidis was cultured on TSA slants at 37°C. The inocula of *S*. Enteritidis were transferred to a 15 ml tube and incubate for 24 hr, and 1 ml of culture were transferred to 9 ml saline solution (NaCl, 0.85%) to a concentration of 10^3 and 10^1 CFU/ml according to the Mac Farland turbidity standard and were used to artificially spiked the cucumber and lettuce samples that were then added to the egg sandwiches.

Raw vegetables samples preparation

Lettuce and sliced cucumber samples were washed under running distilled water and were spiked with 1.0 ml of the 10^3 and 10^1 CFU/ml *S*. Enteritidis culture and let to dry in a laminar air flow. The spiked lettuce or cucumber samples were then added to the egg sandwiches and were incubated at room temperatures and at 4°C. Samples were analysed at 1 hr intervals by placing the samples into sterile plastic bag containing 90 ml of Buffered peptone water (BPW). The mixture was gently homogenized for 60 s in a stomacher and aliquots of 0.1 ml were then spread plated on CHROMagar. The plates were then incubated at 37°C for 24 hr. The *S*. Enteritidis colonies on the CHROMagar plates were counted using a colony counter.

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Results and Discussions

According to the data analysis of the colonies observed on the CHROMagar, *S.* Enteritidis isolate revealed the ability to grow at both temperatures tested. An obvious increase in growth was observed within the first hour mark after the sample spiked with 10^3 cells were incubated at both temperatures, whereas growth was observed after one hour for the samples spiked with 10^1 cells incubated at both temperatures (Figures 1, 2, 3 and 4).

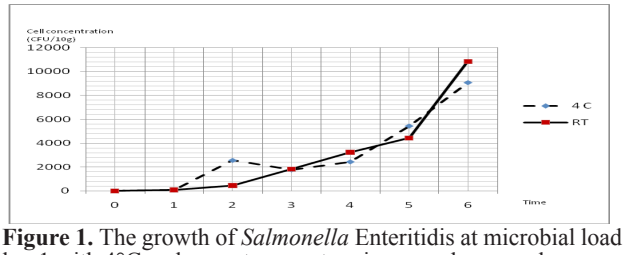

Figure 1. The growth of *Salmonella* Enteritidis at microbial load log 1 with 4°C and room temperature in cucumber sample as raw salad patties

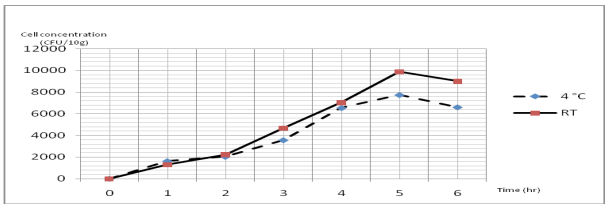

Figure 2. The growth of *Salmonella* Enteritidis at microbial load log 3 at 4°C and room temperature in cucumber as raw salad patties

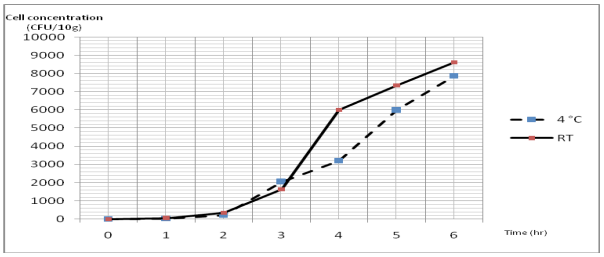

Figure 3. The growth of *Salmonella* Enteritidis microbial load at log 1 at 4°C and room temperature in lettuce as raw salad patties

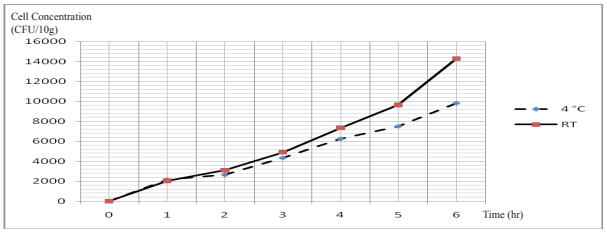

Figure 4. The growth of *Salmonella* Enteritidis microbal load log 3 at 4°C and room temperature in lettuce as salad patties

Salmonella organism including *S.* Enteritidis on egg shells can die rapidly but its survival is enhanced by the high relative humidities and low temperature during storage (Lancaster and Crabb, 1953; Baker, 1990). Therefore, the possibility of the inoculation of even a small amount of *S.* Enteritidis into an egg sandwiches from contaminated raw vegetables used can brings about changes that could facilitate the growth of *Salmonella* organism. According to this study, the growth of the *S.* Enteritidis is dose dependent and that their numbers continued to increase even after 6 hours of incubation. In this study, the 6 hours was selected to reflected the holding time for home made sandwiches served during gathering, small party or even in school cafeterias. Thus, it is important to ensure that the initial load of *S.* Enteritidis must be kept to the minimum since most consumers prefer to add-on raw fresh vegetables such as lettuce, cucumber and tomatoes into the egg sandwiches.

From the data obtained, it is obvious that the *Salmonella* Enteritidis cultures were still growing even at 4°C. It has been reported that *Salmonella* can survive in foods for more than 28 days at 2-4°C in a variety of vegetables such as green beans cabbage salad, beefs, carrots and tomatoes (ICMSF, 1996). Steven *et al*. (2004) reported that the growth of *Salmonella* was recorded in broth at 5.9°C, and as low as 5.2°C and at 4°C even on the plates. Hence, data from this study indicates *Salmonella* can grow even at low temperatures as low as 4°C. Microbial loaded with 10^1 CFU/g shows that *Salmonella* grows slowly but is growing rapidly at room temperature with 10^1 to 10^3 CFU/ g (Figures 1, 2, 3 and 4).

In food microbiology, much effort has been directed at defining limited values, for the growth with single factor such as temperature in wide range of microbes. The optimum temperature for *Salmonella* Enteritidis growth is 35-37°C with most of the strains grow up to 45°C- 47°C though at a reduced rate. For example, *S.* Enteritidis PT 4 inoculated into commercial mayonnaise is destroyed more rapidly when mayonnaise is held at 20°C than held at 4°C (Lock *et al.*, 1994). The low temperature is suitable to maintain the culture for Salmonellae as it can survive for long periods in frozen food which will slow down the growth of *Salmonella* rather than room temperature.

However, this study, shows that the human health risk associated with the consumption of egg sandwich containing raw vegetables is low. The infectivity of *Salmonella* varies with strain, the food vehicle, ages and health status of the patients and thus there is always a necessity for the consumers to be alert and be more concern on the safety of their foods. Bryan (1988), reported that about 10^5 colony performing units was near the minimum number of cells needed to infect healthy adult, whereas other researchers reported that the dose required to cause infection by the *Salmonella* toxin is approximately 10^3 viable

organisms orally for mice to excess of 10^{10} for adult cattle (Hall *et al*., 1978; Hall and Jones, 1979; Lax *et al*., 1995) and 10^7 viable organism are normally required to initiate human gastroenteritis (Blasser and Newman, 1982).

Lettuce and cucumber were used as indicator for *Salmonella* Enteritidis to grow in the eggs sandwiches as these vegetables have been reported to be naturally contaminated. For example, Meldrum *et al*. (2000) reports on the overall contamination of lettuce at 37.5% from the total of 1213 samples of ready-to-eat salads vegetables analysed, and from the perspective of the microbiological safety of salad vegetables, 67/1213 samples (5.5%) of the salad cucumber were contaminated by *Salmonella* spp. in addition to the high proportion of unsatisfactory result for both *E. coli* (6.0%) and *S. aureus* (4.5%). This was further corroborated by another study by Abougrain *et al*. (2009) who reported that cucumber is one of the fresh vegetables which were contaminated significantly.

From the data obtained in this study, it is advisable for consumers to consume freshly prepared sandwiches as most contaminating bacteria will increase with the age. Preventive action such as keeping the salad refrigerated, washing of hands before handling salad and keeping the salad away from raw meat to prevent cross contamination. Prior to using fresh or unpackaged lettuce in sandwiches, remove the outer leaves and wash it thoroughly under running water. Where possible, wash salad greens with a veggie wash surfactant to thoroughly remove remaining dirt, oils and pesticide residues although it does not kill bacteria. These preventive measures can be taken to minimise the numbers viable organism on the fresh produce. In conclusion, proper food handling practices are not just for home practice, but also for the whole food chain. The knowledge about time course for growth may contribute to improve the safety of sandwiches containing eggs and raw vegetables as ingredients.

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