

Survival of *Escherichia coli* 0157:H7 in Kunun-zaki at different storage temperature

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Abstract: *Escherichia coli* 0157:H7 has emerged as a new pathogen of public health importance and currently appear to occur worldwide causing different type of infectious diseases ranging from haemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenic purpura among other infections. This study was carried out to investigate the growth and survival of this organism in Kunun-zaki at different temperatures. 0.1ml of 0.5McFarland standard of *E. coli* 0157:H7 was artificially inoculated into preautoclaved kunun-zaki and stored at refrigerating and room temperatures. The enumeration of these organisms was carried out using standard microbiological techniques at 24 hours intervals. The pH and the titrable acidity were also determined as described by the A.O.A.C methods. Counts of *E. coli* 0157:H7 decreased by approximately 8log units from 8.4log units to 5.10 log unit at 4°C and 5.76 log unit at 27°C. The cell decreased rapidly at a death rate of 0.1520 and 0.230 at both 4°C and 27°C respectively. This indicates that at a specific time, the numbers of cells in kunun zaki were decreasing by 15.2% and 23.0% at refrigerating and at room temperatures. Also, there seems to be a direct relationship between high titrable acidity, low pH and the high death rate observed at room temperature. This study therefore showed that *E. coli* 0157:H7 cannot grow but can only survive for a period of 3.28 days at 4°C and 1.64 days at room temperatures in the kunun-zaki samples used in this study [B.T.Thomas, A. J. Adeleke, O.D.Popoola and O.O. Adebayo. **Survival of *Escherichia coli* 0157:H7 in Kunun-zaki at different storage temperature.** *Researcher* 2012;4(8):13-16]. (ISSN: 1553-9865). <http://www.sciencepub.net/researcher>. 3

Key words: Survival, *Escherichia coli*, Kunun-zaki, Temperatures

1. INTRODUCTION

Escherichia coli 0157:H7 is a bacterium that was first recognized as a food borne pathogen in 1982 when it was associated with two food related outbreaks of haemorrhagic colitis in the United States (Riley *et al.*, 1983). It currently appears to occur worldwide and has three different manifestations; haemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenic purpura (Doyle and Palhye, 1989; Doyle, 1991). In recent years, *E. coli* 0157:H7 and other Shiga-like toxin producing strains have been reported to be transmitted via foods and caused diseases which include bloody diarrhea (Reiman and Cliver, 1998) and other forms of gastroenteritis (Venugopal *et al.*, 2001). Gastroenteritis ranks with respiratory tract infection as the most common infectious disease syndrome of humans (Thomas *et al.*, 2012). Approximately five billion episodes of diarrhea occur worldwide annually accounting for 15-30 percent of all death in some countries (Flint *et al.*, 2005). Kunun-zaki is a traditional fermented non alcoholic beverage that is widely

consumed in Nigeria (Oshoma *et al.*, 2009) with an immense social, economic, nutritional and medicinal benefits to the numerous consumers (Akaoma *et al.*, 2006, Gaffa and Ayo, 2002, Omonigbo and Osobor, 2002). However, the practices associated with its production may exacerbate microbial proliferation (Ogiehor nad Ikenebomoh, 2005). Observations have shown that *Escherichia coli* inoculated into acidic foods may remain viable for few hours at a favourable temperature and up to 1-8 days while refrigerated (Oshoma *et al.*, 2009). This research was carried out to investigate the growth and survival of *Escherichia coli* 0157:H7 in Kunun-zaki at two storage temperature conditions commonly adopted by kunun-zaki sellers.

2.0 MATERIALS AND METHODS

Culture Collection: *Escherichia coli* 0157:H7 was collected from Nigeria institute of Medical Research Yaba, Lagos. This organism was maintained on nutrient agar (Biolab, Germany) slants at 4°C.

2.1 MICROBIOLOGICAL ANALYSIS

The growth and survival of *E. coli* 0157:H7 in kunun-zaki at different temperatures of storage was determined as described by Thomas *et al.* (2012). Prior to inoculation, the kunun-zaki samples were autoclaved at 121°C for 15 minutes and 10ml of each sterilized sample was placed in a pre-sterilized Mac-Catney bottles. Then 0.1ml of the 0.5MCFarland standard of *Escherichia coli* 0157:H7 was inoculated into the MacCatney bottles containing each of the samples. These preparations were mixed by shaking and then incubated at refrigerating and room temperatures. Sampling was carried out at 24 hours interval for five days. 2ml portion of each incubated sample was thoroughly mixed with 18ml of sterile 0.1% (v/v) peptone water and then homogenized. A serial ten fold dilutions were prepared in peptone water and 1ml from each dilution was plated on Eosin methylene blue Agar using Miles and Misra technique. The plates were incubated aerobically at 37°C for 18-24 hours and then the number of colony forming unit per ml of sample was calculated.

2.2 STATISTICAL ANALYSIS

The results were statistically evaluated using SPSS version 19. Independent student t test was used for comparing the specific death rate, pH, titrable acidity and survival period of *E. coli* 0157:H7 per day in kunun-zaki at refrigerating and at room temperatures. The death equation was also used to calculate the length

of survival of *Escherichia coli* 0157:H7 using the equation below:

$$X_t = X_o \cdot e^{-kdt}$$

Where:

X_t = Final concentration of viable cells

X_o = Initial concentration of viable cells

3.0 RESULTS

Table 1 depicts the specific death rate of *Escherichia coli* 0157:H7 in kunun-zaki at both refrigerating and room temperatures. The strain of *E. coli* 0157:H7 used in this study was more adaptable to refrigerating temperature (4°C) than the room temperature as they decreased by approximately 8 log units from 8.4 log units to 5.10 log units at 4°C and 5.76 log unit at 27°C. The cells decreased rapidly at a death rate of 0.152 and 0.230 for both refrigerating and room temperatures respectively. The interpretation of these death rates is that at a particular time, the number of cells at 4°C and 27°C decreased by 15.2% and 23% per total number of cells at that points. The storage temperature was also found to have a significant impact on the final pH and titrable acidity of kunun-zaki and consequently on the specific death rates and the survival period of *E. coli* 0157 H7 in kunun-zaki (table 2).

Table 1. The specific death rates of *E. coli* 0157:H7 in kunun-zaki circulating in Sagamu, Ogun State, Nigeria at different storage temperatures

Temperatures	Specific death Rate per day (d^{-1})	Specific death Rate per hour (h^{-1})
4°C	3.64 ± 0.001	0.152 ± 0.000
27°C	5.52 ± 0.010	0.23 ± 0.000

Table 2. Comparative study of specific death rate, titrable acidity and survival period at 4°C and 27°C for *Escherichia coli* 0157:H7 in kunun-zaki

Parameters	Storage temperatures		t value	pvalue
	4°C	27°C		
Specific death rate (h^{-1})	0.152±0.00	0.23±0.00	-8.5	<0.05
pH	5.16±0.13	3.22±0.11	19.7	<0.05
Titrable acidity	5.9±0.90	7.4±0.00	2.49	<0.05
Survival period per day(s)	3.28±0.45	1.64±0.00	6.3	<0.05

4.0 DISCUSSION

The presence and survival of *Escherichia coli* 0157: H7 in food is of public health concern (Thomas *et al.*, 2012) because of its association with hemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenic purpura (Riley *et al.*, 1983; Doyle

and Palhye, 1989; Doyle, 1991). The artificial inoculation of food with gastroenteritis pathogens and the subsequent storage at 4°C and 27°C were done to mimic post preparation contamination and the popular storage temperature of the food (Thomas *et al.*, 2012). Our choice of kunun-zaki was because this traditional

fermented non alcoholic beverage is widely consumed in Nigeria without any religious discrimination or superstition (Osuntogun and Aboaba, 2004). The ability of *Escherichia coli* 0157:H7 to adapt more to refrigerating temperature (4°C) than room temperature (27°C) may be due to a relative high pH and low titrable acidity of kunun-zaki at 4°C as observed in our study. This finding also corroborates that of Oshoma *et al.* (2009). Generally, bacteria do not usually survive in an acidic medium (Odunfa, 1998). The survival of *Escherichia coli* 0157:H7 for a short period of time may be due to the presence of some medicinal plants such as garlic that are also part of kunun-zaki composition. Other mechanisms that might also contribute to the decline in the population of *Escherichia coli* 0157:H7 are the production of bacteriocin, hydrogen peroxide, and ethanol by the fermentative microorganisms (Ogwaro *et al.*, 2002) which are known to exhibit antagonistic activities against a wide range of pathogenic and spoilage microorganisms (Ouweland, 1998). The apparent effect of storage temperature on the survival of *Escherichia coli* 0157: H7 in kunun-zaki was observed as the organisms survived longer at 4°C than at room temperature (27°C). This observation may be linked to a faster fermentation rate at room temperature leading to acidification of the kunun-zaki and consequently making the kunun-zaki uncondusive for the *Escherichia coli* 0157:H7 (Oshoma *et al.*, 2009). The study showed clearly that *Escherichia coli* 0157:H7 do not grow in kunun-zaki at 4°C and 27°C. This is because growth in real sense only refers to an orderly increase in the number of individual cells making up a population or culture and not increases in size that results when a cell takes up water or deposits lipid or polysaccharide (Brooks *et al.*, 2002). This finding contradicts the findings of Oshoma *et al.* (2009) who reported that *Escherichia coli* 0157:H7 grew in kunun-zaki. Perhaps this may be as a result of regional differences in the compositions of kunun-zaki as some preparations may not contain enough medicinal spices to inhibit the growth of the *Escherichia coli* 0157:H7. The specific survival period of *Escherichia coli* 0157:H7 at 4°C and 27°C were found to be 3.28 days and 1.64 days respectively. This result depicted that *Escherichia coli* 0157:H7 can only survive for 3.28days which is seventy eight hours and seventy two minutes (78hours and 72minutes) at 4°C and 1.64days (39hours and 36 minutes) at 27°C in kunun-zaki. This

observation may be linked to the increased level of lactic acid bacteria and the rapid decline of pH at room temperature as a result of production of organic acids which played a major role in the inhibition of the spoilage organisms. It is therefore important to give room for adequate fermentation of kunun-zaki before consumption. This work disclosed that storage temperature influenced the survival period of *Escherichia coli* 0157:H7 in kunun-zaki. It also demonstrated that the antagonistic activity of LAB and consequently the effect of low pH on the survival of this organism were much more enhanced at high temperatures.

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