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Survival and Acid Tolerance of Shigatoxigenic Escherichia coli (STEC) During Frankfurter Sausage Storage

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Abstract

This study was carried out to understand how sausage matrices affect the survival and acid tolerance of STEC O157 and O26. STEC O157 and O26 were inoculated on sausage surface approximately 5 log. After inoculation sausages were vacuum packed and stored at 4°C. Pathogen counts and synthetic gastric fluid (pH 1,5) experiments were conducted on day 0, 15 and 30 of the storage. Three trials were conducted for each pathogen separately. Both serogroups had viable counts on sausage during storage, STEC O26 count decreased about 1log and O157 about 3log during storage. At the end of the storage both O26 and O157 were viable on the sausage surface 4.59 log and 2.54 log respectively. For acid survival experiments pathogen counts were obtained on 30th, 60th and 90th minute of synthetic gastric fluid (SGF) exposure. Our results show that O26 endured acid stress longer than O157 during SGF experiments throughout storage of frankfurters. The results of this study may support the idea that some non-O157 STEC strains might be more resistant to acid stress than O157 STEC but further studies should be conducted before drawing a conclusion.

Keywords: Frankfurter, STEC, survival, acid tolerance, synthetic gastric fluid.

INTRODUCTION

Shigatoxigenic E.coli (STEC), are important food-borne pathogens that are linked to serious human diseases such as heaemorrhagic colitis and haemolytic uraemic syndrome [1]. These pathogens produce shiga-like toxin also known as verotoxin. Most known member of this pathogenic group is E.coli O157. Shiga-toxin producing other E.coli are commonly referred as non-O157 STECs [2]. The main contamination sources of these pathogens are ruminants, particularly cattle and its products [3]. STEC O157 has been an important pathogen for the food industry.

Non-O157 STECs are more difficult to isolate and most of the laboratories do not conduct analysis to identify them [2]. Due to those reasons there is less information on the prevalence of non-O157 STECs. In regards of virulence some non-O157 STECs; for example outbreak strain O104:H4 in Germany (2011); can be just as dangerous as O157. Non-O157 serotype O26 is the second most prevalent serotype of STEC. Most of the data on these pathogens were obtained from the studies on agar / broth mediums. Since many variables in the food matrices can influence the behavior of these pathogens, there is need for the studies that monitor these pathogens on specific food matrices.

Taking into account that the main source of these bacteria is meat and its products, it is of high importance that the risky meat products are put under the scope. When examining the disease mechanism of E.coli strains acid survival is one thing that stands out the most. Acid survival gives these pathogens the ability to easily survive the stomach acidity and cause diseases. The low infectious dose of these pathogens is associated with this ability. The acid tolerance responses of these organisms are triggered in mildy acidic pH (4.0-5.5) [4].

Frankfurter type sausages are meat products that people of all ages and especially children and young population like to eat. Even though cooking process greatly reduces the microbial load of frankfurter sausages, recontamination might occur usually after cooking. In Turkey, Frankfurter sausages are usually consumed without any additional cooking in cold salads. This eating habit contributes to infection risk associated from this product. Food-borne pathogens that can survive low pH of the stomach such as E.coli, might cause infection via sausages.

In the light of this information, this study aimed to assess the survival ability of O157 and O26 STEC during storage and during synthetic gastric fluid (SGF) exposure of emulsion sausage.

MATERIALS AND METHODS

SBacterial cultures were prepared from agar slants (previously activated from frozen cultures) and passaged three times before use. O157 culture was prepared as mix of two strains (ATCC 43895 and ATCC 35150). Equal amounts of these strains were mixed for O157 inoculum. O26 serogroup was obtained from Istituto Superiore di Sanita (ISS) (Italy).

On the first day of experiments, 18h culture of each pathogen was centrifuged and washed twice. Then the pellet was resuspended in 300ml sterile saline solution. Sausages were dipped in the mixture and stirred gently for 2 minutes to allow the bacteria to attach on the surface (Figure 1). After that the sausages were immediately vacuum packed and stored at 4°C until analysis. Sausages used for this study were provided from local markets on the first day of their arrival and brought to laboratory at 4°C.

On days 0, 15 and 30 of storage microbiological analyses were conducted. For this, 25 g of the sausage samples were added to 225ml of peptone water and macerated for 2 minutes in a Stomacher. The homogenate was serially diluted and 0.1 ml of dilutions was plated onto Sorbitol MacConkey Agar then incubated at 37°C for 24h (start of SGF exposure, minute 0).

SGF was prepared according to Beumer,et.al. [5], briefly; proteose peptone (8.3 g/liter; Difco), d-glucose (3.5 g/liter) NaCl (2.05 g/liter), KH2PO4 (0.6 g/liter), CaCl2 (0.11 g/liter) and KCl (0.37 g/liter) were mixed in deionized water and autoclaved. Ox bile (0.05 g/liter), lysozyme (0.10 g/liter), and pepsin (0.0133 g/liter) was filter sterilized and aseptically mixed. pH of SGF was adjusted to 1.5 with HCl.

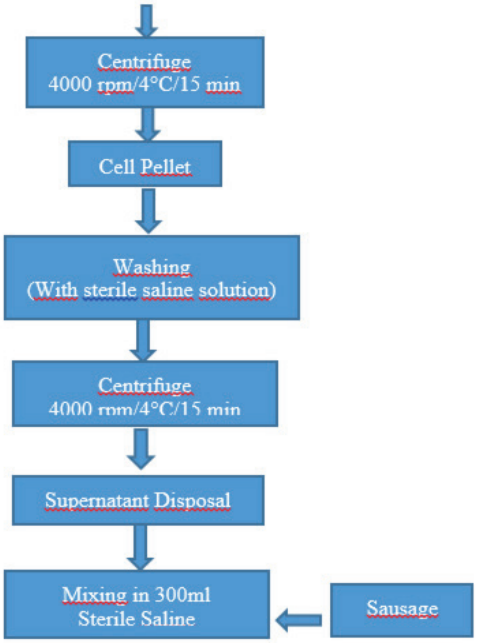

Figure1: Culture preparation and Inoculation

On each SGF exposure trial 20g of sausage was mixed with 120ml of SGF with a stomacher for 2 minutes that after 30, 60 and 90 minutes of exposure the microbial analyses were conducted. The amount of SGF was determined by preliminary experiments in order to keep the pH below 2.5 after 90 minutes of exposure. For synthetic gastric fluid (SGF) exposure experiments 0.1 ml inoculum was plated on SMAC at 30, 60 and 90 minutes of exposure and the plates were incubated at 37°C for 24h. Three trials were conducted for each pathogen and duplicate results were obtained from each plating.

Statistical Analysis

The numbers of bacteria are converted to log10 cfu/g. Then the data were subjected to Analysis of Variance (ANOVA). The means were separated by Fisher's least Square Differences method according to the General Linear Models (GLM) for a significance level of 0.05 [6].

RESULT AND DISCUSSION

Viability of STEC O26 decreased about 1 log during storage (Table 1, Figure 2). On days 0 and 30 of the storage, viable counts decreased significantly in the first 30 minutes of SGF exposure but a tailing effect was observed after 30 minutes. On day 15, viable counts after 60 and 90 minutes of exposure was below the detection limit (Table 2, Figure 3,4,5). But the pathogen seems to recover on day 30, surviving synthetic gastric fluid exposure after 60 and 90 minutes of exposure. STEC O157 showed approximately 3log decrease during storage (Table 1, Figure 2). Viable counts decreased on day 15 but no significant decrease was observed on day 30 (Table 3, Figure 2,3,4).

Table 1: Viability of STEC O26 and O157 during 30 days of storage (log cfu/g ±SD)

Days	O26	O157
0	5.54±0.19^{a}	5.36±0.29^{a}
15	4.89±0.34^{ab}	3.49±1.04^{b}
30	4.59±0.38^{b}	2.54±1.93^{b}

* Same letters in the columns indicate no statistically significant difference was observed

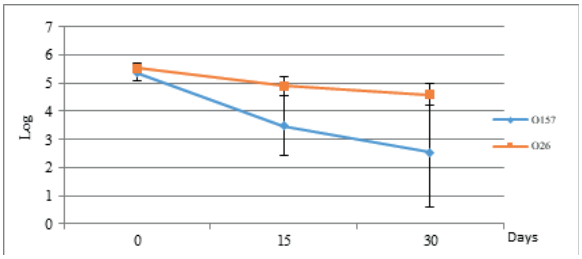

Figure 2: Viability of STEC O26 and O157 During Storage

On the first day of storage STEC O157 could be recovered after 60 minutes of SGF exposure but the count decreased below the detection limit at 90 minutes of exposure. On the 15th day of storage the pathogen showed no viability after SGF exposure. However on the 30th day, O157 survived 30 minutes of SGF exposure but could not be recovered at 60 minutes of exposure.

Table 2: Viability of STEC O26 during SGF Exposure During 30 Days of Storage (log cfu/g ±SD)

	0Min	30 Min	60 Min	90 Min
0	5.54±0.19^{ax}	3.34±0.1 ^{bx}	2.95±0.68^{bcx}	2.12±1.26^{cx}
15	4.89±0.34^{axy}	1.38±0.94^{by}	<0.84	<0.84
30	4.59±0.38^{ay}	1.51±1.14^{by}	1.30±0.82^{by}	1.35±0.89^{by}

**Same letters in the columns (x,y,z,t) and in the rows (a,b,c,d) indicate no statistically significant difference was observed*

Table 3: Viability of STEC O157 during SGF Exposure During 30 Days of Storage (log cfu/g ±SD)

	0 Min	30 Min	60 Min	90 Min
0	5.36±0.29^{ax}	1.92±1.77^{bx}	1.26±1.08^{b}	<0.84
15	3.49±1.04^{y}	<0.84	<0.84	<0.84
30	2.54±1.93^{ay}	1.06±0.63^{ax}	<0.84	<0.84

** Same letters in the columns (x,y,z,t) and in the rows (a,b,c,d) indicate no statistically significant difference was observed*

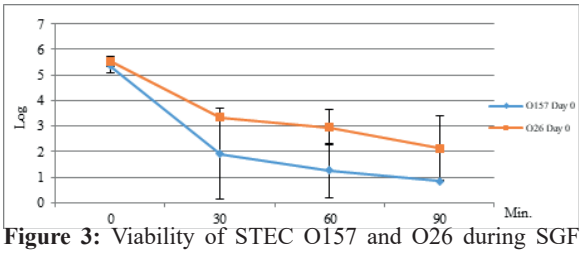

Figure 3: Viability of STEC O157 and O26 during SGF Exposure Day 0 of the Storage

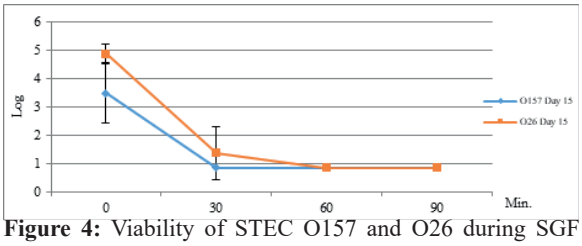

Figure 4: Viability of STEC O157 and O26 during SGF Exposure Day 15 of the Storage

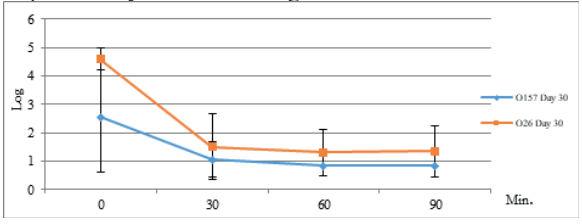

Figure 5: Viability of STEC O157 and O26 during SGF Exposure Day 30 of the Storage

The viability of STEC O26 was higher than O157 during both sausage storage and SGF experiments. O26 survived about 1-2 log after 90 minutes of SGF exposure (except on day 15), while O157 couldn't survive after 90 minutes of exposure on each storage day. The ability to survive SGF of both pathogens decreased on day 15 and then increased again on day 30.

Bergholz&Whittam [7], compared acid resistance of O26:H11, O111:H8 and O157:H7 serotype strains and concluded that O157:H7 had superior ability to survive simulated gastric acidity. They mixed SGF with baby food and inoculated this mixture with stationary phase cultures. Berry et.al. [8], compared acid resistance (AR) of O157 and non-O157 isolates. At the end of 6 hours in Brain Heart Infusion broth (pH 2.5), even though they didn't find any significant difference of survival rates; they reported O157 strains had higher percentage of injured cells than non-O157 strains.

In other respects, Miszczycha et.al. [9], reported E.coli O26:H11 had significantly higher survival rate than O157:H7 when experimentally contaminated cheese subjected to artificial digestion. Elhadidy& Mohammed [10], also reported that O26:H11 had better ability to survive acidic pH than O157:H7 at pH values tested (4.5 and 6.5).

CONCLUSION

Comparing the results of acid survival studies is problematic. Researchers use different methods and mediums to obtain results. In general researchers make medium (minimal versus complex), growth phase (stationary phase cells versus log phase cells) and various temperature or pH comparisons in their research. STEC has various acid resistance systems that are induced under different conditions. Also there is strain-based difference on the acid survival rates of STEC. In addition to that

acid resistance systems of STEC O157 and non-O157 might differ. Therefore it is important to also report results on actual specific food systems that might affect the acid survival systems induced by these bacteria.

Results of this study showed STEC O26 preserved its acid resistance ability longer than STEC O157 during SGF experiments. Therefore it can be speculated that STEC O26 might have better acid survival ability than STEC O157, on sausage surface. But further studies should be conducted to be able to draw a conclusion like that.

Emulsion sausages can be consumed without any additional cooking (e.g. in cold salads) therefore the food safety risk associated with these products increases. Especially since the infectious dose of STEC can be very low. Further studies need to be conducted in order to better understand the behavior of these pathogens on certain food matrices and the factors affecting their acid survival ability.

Acknowledgements

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