As. J. Food Ag-Ind. **2012**, 5(06), 567-578

OPEN ACCESS

Asian Journal of Food and Agro-Industry

ISSN 1906-3040

Available online at www.ajofai.info

Research Article

Survival of shiga toxin producing *E. coli* in Nham and effectiveness of thermal inactivation by microwaving

W. Chantarapanont[1], S. Nitisinprasert[2] and J.F. Frank[3]

[1]Department of Product Development, Faculty of Agro-industry, Kasetsart University, Chatuchak, Bangkok 10900, Thailand.

[2]Department of Biotechnology, Faculty of Agro-industry, Kasetsart University, Chatuchak, Bangkok 10900, Thailand.

[3]Department of Food Science & Technology, University of Georgia, Athens GA, USA

Email: walairut.c@ku.ac.th

Abstract

A study was conducted to determine if fermentation conditions of Nham were effective against *E. coli* O157:H7 and three non-O157:H7 shiga toxin producing *E. coli*, O8, O111 and untypeable O strain, comparing with non pathogenic *E. coli*, E780, as a negative control. Nham was produced using starter cultures V3 (*Pediococcus acidilactici* and *Staphylococcus xylosus*) provided by Vicchi Consolidated Co., Ltd. Each of five strains of *E. coli* were separately inoculated to unfermented Nham giving initial population of ca 2 x 10^6 CFU/g and fermented at 35°C for 3 days and stored at 4°C for up to 20 days after that. All strains except O157:H7 were increased by 1 log for the first day of fermentation and decreased by 1 to 2 log after Nham fermentation for 3 days. After storing inoculated Nham at 4°C, 3 strains of non-O157:H7 slowly decreased only 0.5-1 log till 20 days. The effectiveness of thermal activation was determined using capillary tube method. Thermal Death Time (D value) at 58°C in acidified TSB (pH 4.6) of all strains of non-acid adapted *E. coli* (TSB) was higher than acid adapted *E. coli* (TSB pH 5.8). Non acid-adapted and acid-adapted *E. coli* O111 showed the highest D value (3.31 and 1.23 min, respectively) at 58°C while *E. coli* O157:H7 showed the least D value (1.96 and 0.37 min, respectively). Results showed that heating by 900 watt microwave at initial temperatures of 28°C for 60 seconds could not destroy any shiga toxin producing *E. coli* (ca. 1 x 10^5 CFU/g) in Nham. *E. coli* O157:H7 could be destroyed after heating with microwave up to 90 seconds, but not all *E. coli* O111. Microwaving Nham might not be a productive method for safely cooking Nham.

Keywords: fermentation, TSB, O157:H7, non-O157:H7, D-value, pork, Thailand.

Introduction

Nham is a Thai fermented pork sausage, generally regarded as a traditional food and very popular among Thai people. Nham basically contains minced pork, shredded pork skin, 2-3% NaCl, cooked rice, garlic and 100-125 ppm of sodium nitrite [1]. After mixing all the ingredients together, they are wrapped tightly in banana leaves or plastic bags, fermented at room temperature for about 3-4 days and served with or without cooking after that. Coliform, fecal coliform and *Escherichia coli* in Nham from Thai local markets could be as high as 10^8 cfu/g during the first day of Nham fermentation and last till the 35th date of fermentation, but for good taste, Nham is usually fermented for 4-5 days at room temperature and kept in a refrigerator after that [2]. To destroy parasites, meat is frozen before processing and lactic acid fermentation is employed to control bacterial pathogens from growing and producing toxins [3]. These fermented sausages are considered safe due to the intrinsic and extrinsic factors used in the processing system [4]. This fermented product is always eaten as raw food because the cooking destroys its fermented flavour. However, consumption of Nham raises questions regarding the manufacturing practices and safety of the product. There are several pathogenic bacteria such as *Salmonella* and *E. coli* O157:H7 which can grow or survive at low pH and are originally found in pork and meat [5]. So Nham can be categorized as a high risk product and for which it is highly recommended to serve only after cooking to ensure safety. Methods of cooking are various including frying, grilling and microwaving.

Somathiti [6] examined four hundred and fifty samples from nine Nham producers in Bangkok. Coliform, fecal coliform and *E. coli* were found 262.5 cfu/g at the fifth day of fermentation which 56 out of 450 samples were found to contain Salmonellae but pathogenic *E. coli, E. coli* O157:H7, were not examined in this survey. *Escherichia coli* is a gram-negative, facultative anaerobe and is considered to be a part of the normal microflora in the intestines in humans as well as in animals. However, some strains can cause diseases in various organic systems. Serotype O157:H7 is a rare type of *E. coli* linked with the production of one or more toxins known as verotoxin or shiga-like toxin which cause hemorrhagic colitis, abdominal cramps and bloody diarrhea in humans. They are similar to the toxin produced by *Shigella dysenteriae* [7]. In limited cases, this illness has the potential to progress to life-threatening hemolytic uremic syndrome, the leading cause of renal failure in children, and thrombotic thrombocytopenic purpura affecting adults [8]. Epidemiological investigations demonstrated that cattle, both beef and dairy, pork and deer were principal reservoirs of *E. coli* O157:H7 [9]. There was evidence from many outbreaks that this serotype of *E. coli* possessed the unusual characteristic of acid tolerance [10]. Numerous other less likely contaminated food items have been implicated in outbreaks, including acid or acidified food such as unpasteurized apple cider and apple juice, mayonnaise and mayonnaise-based salad dressings, yoghurt, and salami [8]. Growth of *E. coli* O157:H7 at pH as low as 4.0 to 4.5 has been recorded in broth studies [11]. Organic acids present in processed food and pH reductions brought about by increased production of these acids, e.g., during the fermentation of meat, allow *E. coli* O157:H7 to adapt to acid conditions. The ability to adapt to acidic conditions allows the organism to later tolerate pH levels that would normally inactivate it [12]. This property also contributes to the ability of *E. coli* O157:H7 to survive during the processing and storage of fermented meat [13], which generally has a final pH in the range of 4.5 to 5.5. Acid-adapted or acid-shocked bacterial cells are known to have enhanced protection against subsequent exposure to acidic or other stress environments such as heat stresses. The possibility of acid tolerance imparting cross-protection against heat would have significant implications for the application of heat as a means of eliminating *E. coli* O157:H7 in fermented meat. Although Shiga toxin (Stx)- producing *E. coli* (STEC) belonging to O157:H7 serotype predominates in various parts of the world, infections with non-O157 STEC are now increasingly recognized in many countries [14].

The objective of this research was to determine if acid-adapted STEC (O157:H7 and non-O157) grown in reduced pH growth media (TSB pH 5.8) had an impact on the survival in Nham during fermentation and under refrigeration temperature after fermentation and also to study survival of STEC (O157:H7 and non-O157) in Nham after heat inactivation by microwave.

Materials and Methods

Bacterial culture

Four stains of shiga toxin producing *E. coli*, which were *E. coli* O157:H7 (*stx+, eaeA+)*, *E. coli* serogroup O8 (*stx+, eaeA-*), *E. coli* untypable O serogroup (*stx+, eaeA-)* and *E. coli* serogroup O111 (*stx+, eaeA+),* were used and compared to the non-shiga-toxin-producing strain of *E. coli,* E780 (*stx-,eaeA-),* which was used as a negative control in this experiment. All cultures were purchased from Enteric Laboratory, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand. All strains were stored in 10-15% glycerol at -70°C until used.

Confirmation of virulence markers by multiplex PCR

All strains were confirmed virulence markers by multiplex PCR [15]. One colony of each 24 hr-grown *E. coli* strain which was selected, suspended and boiled in 10 µl of water for 10 min was used as PCR template. Each boiled lysate *E. coli* was subjected to two PCR assays using two multiplex primer sets. The first set was used to identify Enterotoxicgenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC) and Shigatoxin-producing *E. coli* (STEC) which was *stx* (*slt* ½) (F) TTTACGATAGACTTCTCGAC (R) CACATATAAA TTATTTCGCTC and produced PCR product of 228 bp. The second set was used to detect Enteropathogenic *E. coli* (EPEC which was *eaeA* (Intimin) (F) GCTTAGTGCTGGTTT AGGAT (R) TCGCCGTTCAGAGATCGC and produced PCR product of 488 bp. PCR assays were carried out in 25 µl reaction mixtures consisting of 5 µl template DNA, 10xbuffer, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.2 µM of each primer and 1 U *Taq* DNA polymerase. The reaction mixtures were run in a thermal cycler with the following cycling profile: 94°C for 5 min, 25 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1.5 min and primer extension at 72°C for 2 min and a final extension at 72°C for 5 min. The annealing temperature for EPEC PCR was 50°C. Amplified products were resolved by 2% agarose gel electrophoresis and visualized under UV transillumination after ethidium bromide staining.

Study survival of acid-adapted and non acid-adapted shiga toxin producing E. coli in Nham

Preparation of acid-adapted shiga toxin producing E. coli strain

Acidified Tryptic Soy Broth (acidified TSB), which was adjusted to pH 5.8 by adding filtered sterile 1M lactic acid, was used as a growth medium for acid-adapted *E. coli*. Each of 5 strains of *E. coli,* which was individually grown in TSB (pH 7.0) and acidified TSB at 37°C (pH 5.8) for 18 h and transferred (1 ml) to 10 ml of TSB and acidified TSB twice at 24-h interval was used as non acid-adapted and acid-adapted inoculum for study survival in Nham, respectively. Virulence markers of acid-adapted and non acid-adapted shiga toxin producing *E. coli* strains were confirmed by multiple PCR as described above.

Inoculation and Nham preparation

Nham was freshly prepared by using thoroughly mixed ground pork (60%), boiled shredded pork skin (27%), cooked glutinous rice (5%), minced garlic (5%), salt (2%) and a commercial curing agent which was formulated to provide a mixture of polyphosphate, nitrite and colour stabilizer (1%). After that a mixture of *Pediococcus acidilactici* and *Staphylococcus xylosus* bacterial culture (Starter V3; Vicki Consolidate Company, Bangkok, Thailand) which was used as Nham starter culture was added to unfermented Nham batter at the level recommended by the manufacturer (ca. 10^5 CFU/g). Five hundred grams of unfermented Nham was individually inoculated with ca. 2 x 10^6

CFU/g of acid-adapted or non-acid adapted cells of each of 4 stains of *E. coli* producing shiga toxin and 1 strain of *E. coli* which is not shiga toxin producing which was prepared as described above. The cells were mixed in unfermented Nham by thoroughly massaging the mixture by hand for 5 min. Each inoculated Nham was divided into 9 plastic bags (50g each). The mixture was squeezed to the closed bag end, wrapped tightly with rubber bands and left at room temperature (35°C) for fermentation for 3 days and after that stored in refrigerator (4°C) up to 20 days after fermentation.

Microbiological and chemical analysis of Nham

One bag of inoculated Nham was sampled at day 0, 1, 2, 3 of fermentation and after storage at refrigeration temperature (4°C) for 4, 8, 12, 16, and 20 days. Twenty five grams of Nham were taken from a bag and stomached with 225 ml of Trypticase Soy Broth (TSB) for 1 min and serially (1:10) diluted in 0.1 M phosphate buffer saline (PBS), after which 0.1 ml was surface plated on Chromocult® (Merck, Germany) and incubated at 35°C 24 hr to enumerate *E. coli* and also plated on MRS agar (Merck, Germany) and incubated at 35°C 24 hr to enumerate lactic acid bacteria. Control Nham which was not inoculated with *E. coli* was also tested for background *E. coli* at day 0. Presumptive colonies of *E. coli* O157:H7 were screened by the *E. coli* O157:H7 latex agglutination test (Oxoid, England). Randomly selected colonies were confirmed as shiga toxin producing *E. coli* by multiplex PCR as described above. Results reported were the average of duplicate trial of 2 replications.

Titratable acidity and pH were also determined at the same sampling time. A 10-g sample was macerated by Stomacher with 90 ml of distilled water and heated to boil and allowed to cool to room temperature. The homogenate was filtered through Whatman no.1 filter paper. Filtrate was added with a few drops of Phenolpthalein as pH indicator and titrated with 0.1 N NaOH until colour changed. Titratable acidity (TA) was calculated by using the following equation:

$$\%TA = [(\text{Normality of titrant} \times \text{ml of titrant} \times \text{meq wt of acid}) \times 100]/ \text{g of sample}.$$

Titratable activity was expressed as percent lactic acid; the milliequivalent weight of lactic acid is 0.09 [16]. For pH measurement, a 5-g sample was blended with 5 ml of distilled water for 1 min and the slurry measured using pH meter (Consort C830, Belgium). Results were reported in terms of the average of duplicate trial of 2 replications.

Study of thermal inactivation time of acid-adapted and non-acid adapted shiga toxin producing E. coli in acidified TSB

Thermal inactivation time of acid-adapted or non-adapted cells of each of 4 stains of *E. coli* producing shiga toxin and 1 strain of *E. coli* which is not shiga toxin producing were determined according to the procedure described by Chantarapanont *et al.* [17]. Acidified TSB (9.9 ml) which was Tryptic Soy Broth adjusted pH to 4.6 by adding filtered sterile 1M lactic acid was individually inoculated with 0.1 ml of acid-adapted or non-acid adapted cells of each of 4 stains of *E. coli* producing shiga toxin and 1 strain of *E. coli* which is not shiga toxin producing (ca. $5x10^6$ CFU/ml). Each strain was tested separately. After thoroughly mixing, inoculated products were held in an ice water bath for no more than 30 min before injecting fifty µl of the products into sterile glass capillary tube (0.8 to 1.1 mm inside diameter by 100 mm long) using a sterile 1-cm^3 syringe fitted with a 22-guage, 4-in stainless steel needle. Filled tubes were flame-sealed and held in ice-water slurry until immersed in a hot water bath to determine thermal death time (D value) of all test strains. Capillary tubes were placed upright in a test tube rack and immersed in a water bath (Memmert, Germany) at 58°C. Preliminary experiments showed that come-up times over a temperature of 58°C averaged 2 to 4s at the approximate centre of each capillary tube. Inoculated tubes were removed from the water bath at 1 min 30 sec intervals. Tubes were placed in ice-water slurry for 1 to 2 min before analyzing for populations of viable *E. coli.*

Following this, the exterior of each tube was disinfected in 70% ethyl alcohol and dried with sterile absorbent paper. Individual capillary tubes were then transferred to test tube containing 5 ml of sterile 0.1M sterile PBS and crushed finely using a flame-sterilized glass rod. After thoroughly mixing, samples were serially (1:10) diluted in 0.1 M phosphate buffer saline (PBS), after which 0.1 ml was plated on TSA and incubated at 35°C 24 hr for enumerate *E. coli.* Presumptive colonies of *E. coli* O157:H7 were screened by the *E. coli* O157:H7 latex agglutination test (Oxoid, England). Randomly selected colonies were confirmed as shiga toxin producing *E. coli* by multiplex PCR as described above.

Thermal inactivation experiments were conducted in triplicate for each strain and two capillary tubes were used for a replication. The thermal death time (D value) was calculated as the absolute value of the reciprocal of the slope of the survivor curve ($\log_{10}$ CFU/ml versus time). Using a minimum of five heating times, a best-fit line for the survivor curve was calculated and plotted using a personal computer with a Microsoft Excel 2003 program (Microsoft Corporation, Redmond, Wash.). D values were tested for significant differences ($P \leq 0.05$) using SPSS version 11.5 (SPSS Inc., Chicago, IL).

Study of thermal inactivation of shiga toxin producing E. coli in Nham using microwave oven

Nham was prepared and individually inoculated with ca. 1 x 10^5 CFU/g of selected shiga toxin producing *E. coli* which showed the highest D value and *E. coli* O157:H7. Each inoculated Nham was divided into 7 plastic bags (50g each), squeezed to the closed bag end, wrapped tightly with rubber bands and left at room temperature (35°C) for 3 days fermentation. After 3 days, Nham (50g) were aseptically taken out of plastic bags, placed each in the middle of Pyrex 2-cup round with plastic wrap (Corning Incorporated, USA) and reheated with microwave (model R-4A68, 33L, 220 V 50 cycle, 900 Watt, 2,450 MHz, Sharp Corporation, Japan) for 15, 30, 45, 60, 75 and 90s. Twenty five grams of the heated Nham were transferred aseptically to a stomacher bag (Seward, London, England) containing 225 ml of EC broth (Merck) using a sterile spoon. The mixture was pummeled for 1 min at normal speed using the stomacher. One millilitre of the mixture was serially (1:10) diluted in 0.1 M phosphate buffer saline (PBS) and 0.1 ml was surface plated in duplicate on Chromocult®; undiluted samples (0.25 ml in quadruplicate and 0.1 ml in duplicate) were also plated on Chromocult® and incubated at 35°C 24 hr for enumerate *E. coli*. The remainder of the mixture of Nham and EC broth was incubated for 24 h at 35°C. When samples plated on Chromocult® failed to yield presumptive *E. coli* colonies, enriched cultures were then streaked on EMB (Merck) and incubated at 35°C for 24 h. Presumptive *E. coli* were subjected to IMViC test and also randomly selected to confirm as shiga toxin producing *E. coli* by multiplex PCR. Quadruplet thermal inactivation experiments were conducted for each strain.

Results and Discussion

Confirmation of virulence markers by multiplex PCR

PCR amplification of 5 strains of *E. coli* with primer pair of *Stx* (SLT ½) and *EaeA* (intimin) demonstrated that only *E. coli* strains containing *Stx* gene (*stx*+) which were *E. coli* O157:H7, *E. coli* serogroup O8, *E. coli* untypable O serogroup and *E. coli* serogroup O111 showed bands at 228bp (Figure 1) and only *E. coli* strains containing *eaeA* gene (*eaeA*+) which are *E. coli* O157:H7 and *E. coli* serogroup O111 showed bands at 488 bp, while the others showed 3 special bands (Figure 2).

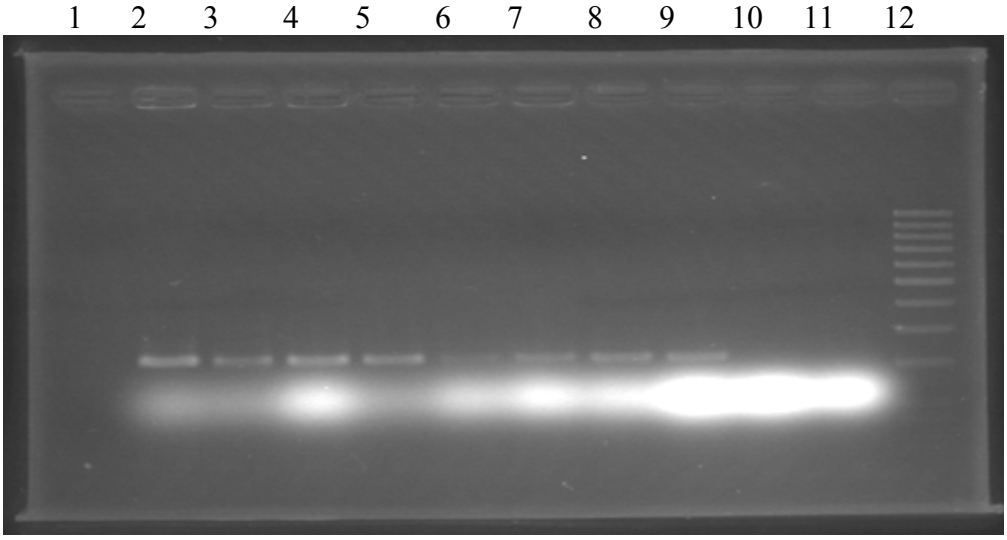

Figure 1. PCR amplification of *stx* from 5 strains of *E. coli* with PCR primer pair of *Stx* (SLT ½).

Lane 1, control; lane 2-3, *E. coli* O157:H7; lane 4-5, *E. coli* O111; lane 6-7, *E. coli* O8; lane 8-9, *E. coli* untypable O serogroup; lane 10-11 *E. coli* which is not shiga toxin producing (negative control); lane 12,100 bp ladder. The resulting PCR amplicons were visualized following electrophoresis with 2% agarose gel containing ethidium bromide. PCR product of primer pair of *Stx* (SLT ½) is 228 bp.

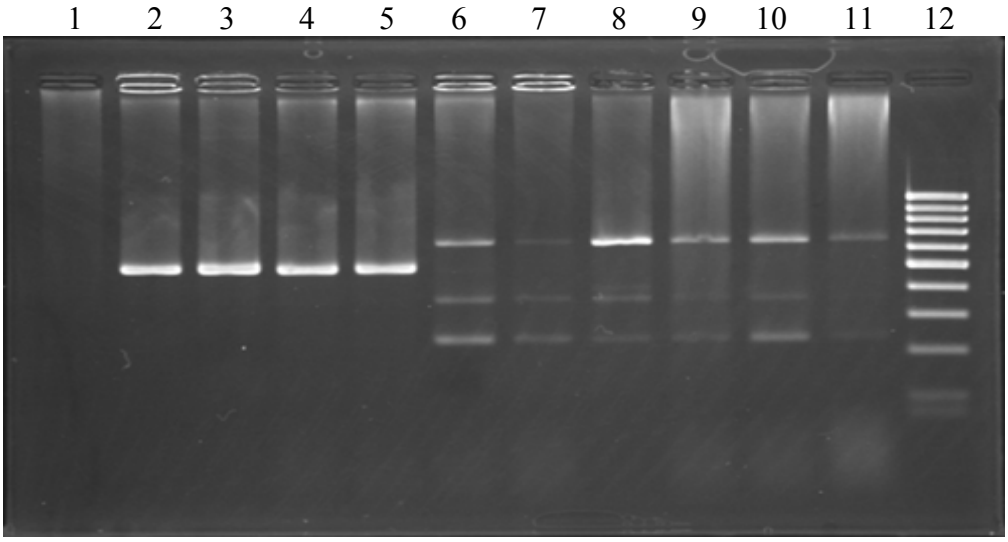

Figure 2. PCR amplification of *eaeA* from 5 strains of *E. coli* with PCR primer pair of *EaeA* (intimin).

Lane 1, control; lane 2-3, *E. coli* O157:H7; lane 4-5, *E. coli* O111; lane 6-7, *E. coli* O8; lane 8-9, *E. coli* untypable O serogroup; lane 10-11 *E. coli* which is not shiga toxin producing (negative control); lane 12,100 bp ladder. The resulting PCR amplicons were visualized following electrophoresis with 2% agarose gel containing ethidium bromide. PCR product of primer pair of *EaeA* (intimin) is 488 bp.

Study of survival of acid-adapted and non acid-adapted shiga toxin producing E. coli in Nham

In this study, commercial starter culture was used since the advantage of using starter cultures gave a shorter fermentation time. The microbial safety of this fermented product depends on the rapid decreasing rate of pH which is due to the presence of starter cultures, carbon sources (cooked rice or sugar) and garlic [1] Growth of lactic acid bacteria reduced the pH after fermentation for 3 days. The pH of Nham dropped from 6.39 to under 4.6 and was stable after that at refrigeration

temperature until 20 days of storage (Table 1). Number of lactic acid bacteria increased to 8 log CFU within the first 24 hours and was stable after that.

Table 1. Titratable acidity (%lactic acid), pH and population of lactic acid bacteria in Nham during fermentation at 35°C for 3 days and at storage temperature (4°C) up to 20 days after fermentation.

	Day	%Lactic acid	pH	Lactic acid bacteria (log CFU/g)
Fermentation day at 35°C	0	3.14	6.39	4.93
	1	3.78	5.41	8.43
	2	6.44	4.68	8.62
	3	7.21	4.57	8.41
Storage temperature (4°C)	4	8.26	4.55	8.27
	8	8.23	4.55	8.34
	12	7.65	4.54	8.43
	16	8.19	4.55	8.48
	20	7.83	4.49	8.07

Glass *et al.* [16] found that when TSB was acidified with lactic acid, *E. coli* O157:H7 grew at pH 4.6 but not at pH 4.5. However, acid and non-acid adapted *E. coli* could survive in Nham after fermentation for 3 days at 35°C (pH 4.57) and up to 20 days at refrigeration temperature with just 1-2 log reduction, except non-acid adapted *E. coli* O157:H7 which decreased for 4 log CFU (Fig 3 and 4). All strains except O157:H7 were increased by 1 log for the first day of fermentation and decreased by 1 to 2 log after Nham fermentation for 3 days. After storing inoculated Nham at 4°C, 3 strains of non-O157:H7 slowly decreased only 0.5-1 log till 20 days of 4°C storage. In addition, after *E. coli* O157:H7 subjected to acid, the survival ability in Nham increased obviously (Fig 4). *E. coli* O157:H7 has been noted for its acid-adaptive and acid tolerance properties in a number of food items and under a variety of conditions [18]. Leyer *et al.* [19] suggested that acid adaptation was important for the survival of *E. coli* O157:H7 in acidic food and should be considered a prerequisite for inocula used in food challenge studies. Strains of *E. coli* O157:H7 also vary widely in their acid tolerance and survival would be expected to differ on studies employing different strains. Other researchers have reported that enteroinvasive and enteropathogenic *E. coli* organisms were significantly more acid tolerant than nonpathogenic strains. From the results of this study, acid adapted or nonacid adapted *E. coli* producing shiga toxin, except *E. coli* O157:H7, compared with nonpathogenic strain (E780) showed the same trend in their survival in Nham. It was only 0.5-1 log difference between acid adapted or non-acid adapted in each strain except *E. coli* O157:H7.

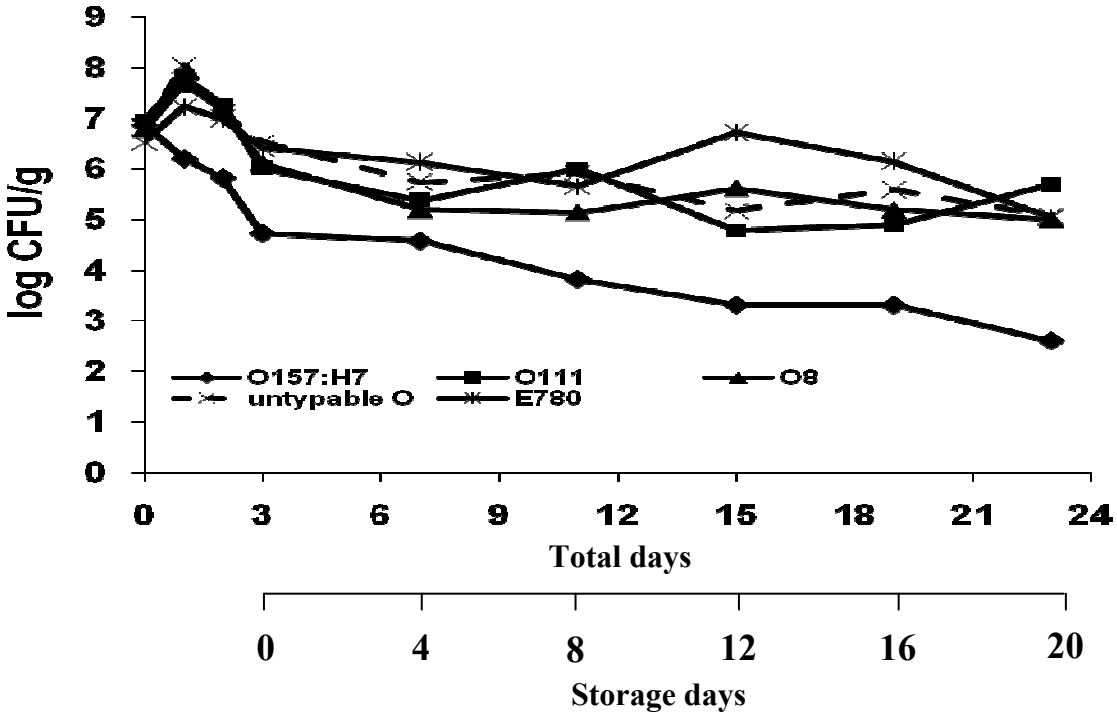

Figure 3. Populations of non-acid adapted *E. coli* in Nham during fermentation at 35°C for 3 days and after storage at 4°C up to 20 days after fermentation.

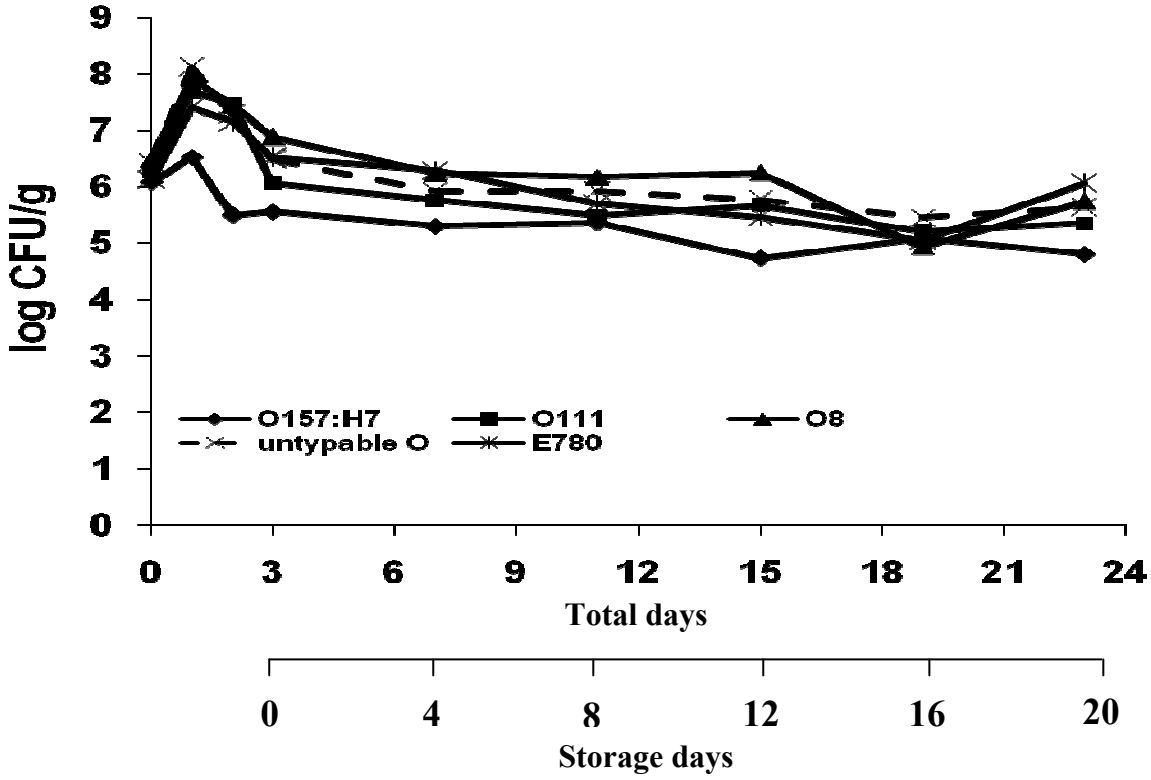

Figure 4. Populations of acid-adapted *E. coli* in Nham during fermentation at 35°C for 3 days and after storage at 4°C up to 20 days after fermentation.

Study of thermal inactivation time of acid-adapted and non acid-adapted shiga toxin producing E. coli in acidified TSB (pH 4.6)

Only non-acid adapted *E. coli* O8 had significantly higher D value ($p \leq 0.05$) than the acid-adapted one, whereas the rest of treatment showed higher trend of D value but they were not significantly different ($p > 0.05$). *E. coli* O157:H7 had the lowest D value in both acid and non-acid adapted with insignificant difference between acid or non-acid adapted ($p > 0.05$). Non-acid adapted and acid-adapted *E. coli* O111 showed the highest D value (3.31 min and 1.23, respectively) at 58°C and significant difference from *E. coli* O157:H7 ($p \leq 0.05$). D value of *E. coli* O157:H7 at 55°C in TSB were ranged between 1.0-25.6 min [20, 21]. Splittstoesser *et al.* [22] showed that D value at 58°C of *E. coli* O157:H7 in apple juice (pH 4.4) was 1.0 min which was not different from D value of *E. coli* O157:H7 in this study. However, acid adapted *E. coli* O157:H7 showed more sensitivity to heat than non-acid adapted. The stress response that gives rise to acid tolerance has not been shown to confer some cross-protective effects against heat stresses which contrasts with Cheville, *et. al.* [18].

Table 2. D values (min) at 58°C of acid adapted and non-acid adapted *E. coli* in acidified TSB (pH 4.6).

E. coli	D value (min) ± S.D.	
	Acid adapted	**Non-acid adapted**
O157:H7 [NS]	0.37 ± 0.02 [b]	1.96 ± 0.62 [b]
Untypable O [NS]	0.66 ± 0.15 [ab]	1.99 ± 0.27 [b]
O8	0.68 ± 0.02 [ab B]	2.74 ± 0.28 [ab A]
E 780 [NS]	0.84 ± 0.19 [ab]	3.12 ± 0.90 [ab]
O111 [NS]	1.23 ± 0.64 [a]	3.31 ± 0.57 [a]

[a,b] Means within the same column with different superscripts showed statistically significant difference ($p \leq 0.05$).
[NS] Means within the same row showed statistically insignificant difference ($p > 0.05$).
[A,B] Means within the same row with different superscripts showed statistically significant difference ($p \leq 0.05$).

Study of thermal inactivation of shiga toxin producing E. coli in Nham by microwaving

From a consumer survey, results showed that Thai people usually microwaving Nham for cooking (data not shown). Thus non-acid adapted ETEC (O111), the highest $D_{58°c}$ (3.31 ± 0.57 min) from D value study, was chosen to study survival in Nham after thermal inactivation by microwaving compared with non acid-adapted ETEC (O157:H7).

Microwave ovens have provided several benefits in terms of speed and convenience; however, there are a lot of disadvantages, such as non-uniformity of heating, edge overheating, soggy texture and lack of browning [23]. Heating by 900 Watt microwave at initial temperatures of 28°C for 60 seconds could not destroy any shiga toxin producing *E. coli* (ca. 1 x 10^5 CFU/g) in Nham. *E. coli* O157:H7 could be destroyed after heating with microwave up to 90 seconds, but not all *E. coli* O111 (Table 3). In addition, Nham tended to overburn after microwaving more than 60 sec. Traditional ways of heat cooking, frying and roasting, that can heat Nham thoroughly are a better choice than using microwaving in order to destroy pathogenic *E. coli* that might be contaminated in Nham.

Table 3. Survival of *E. coli* O111 in Nham when heating with 900 Watt microwave for 90s at initial temperature of 28°C.

Heating time (second)	*E. coli* O157:H7	*E. coli* O111
0	4/4 [b]	4/4
15	4/4	4/4
30	4/4	4/4
45	4/4	4/4
60	4/4	4/4
75	1/4	3/4
90	0/4	1/4

[a]Heating time (second) used in heating Nham in microwave
[b]Number represented number of samples that *E. coli* were positive from 4 replications

Conclusion

E. coli O157:H7 seemed to be more sensitive to pH and heat than other shiga-toxin producing *E. coli*. Acid adaptation of *E. coli* O157:H7 may increase risk in Nham due to its increased survival. All strains of shiga-toxin producing *E. coli* (non O157) did not show significant effect of acid adaptation to increase survival in Nham during storage at 4°C as *E. coli* O157:H7 did. Heat inactivation is still the best way to increase safety in consuming Nham. However, microwaving Nham might not be a good way of cooking Nham.

Acknowledgement

This research was supported by Thai Research Fund (TRF).

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