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The Fate of the Fecal Coliform, *Escherichia coli*, in Baby Foods Stored Frozen

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Abstract: The fecal coliform, *Escherichia coli*, was inoculated (7 $\log_{10}$ cfu g^{-1}) in 3 commercial baby foods, which were subsequently frozen and stored (-18°C) for 26 days. Mathematical model equations were developed describing the effect of freezing and frozen storage on the viable ($Pv = (2.83*10^{-8} + (6.18*10^{-8}*t))^{-1}$) and undamaged populations ($Pud = (1.76*10^{-7} + (4.59*10^{-6}*t))^{-1}$) of *E. coli*. Frozen storage for 1 day reduced the viable and undamaged populations of *E. coli*, in all baby foods, by about 66.0-91.0 and 66.0-99.0%, respectively. At the same time, the level of damaged cells of *E. coli* was the highest in all baby foods. After 26 days of frozen storage the viable, undamaged and damaged populations of *E. coli* declined by about 1.0-3.8 log cfu g^{-1}.

Key words: *E. coli*, fecal coliform, freezing and frozen storage, baby foods

INTRODUCTION

The primary source of the fecal coliform *E. coli* is the intestinal tract of humans and animals. The majority of the *E. coli* in the human intestine is harmless except for 5 pathogenic groups of *E. coli who* cause disease in humans (Kuntz and Kuntz, 1999). One of these groups is Enterohemorrhagic *E. coli* (or EHEC). EHEC produces potent toxins (verocytotoxin or Shiga-like toxin) and can cause severe disease in human (Clarke, 2001). The most important strain among EHEC associated with human disease is *E. coli* O157:H7. Infections by *E. coli* O157:H7 were linked to the consumption of less than 50 organisms and possibly as low as 5 (Armstrong *et al*., 1996). Strains of EHEC are responsible for a range of illnesses which may be severe and sometimes fatal, particularly in infants, young children and the elderly.

The fecal coliform *E. coli* is easily detected by its ability to ferment lactose. It is relatively easier to isolate than some known gastrointestinal pathogens. Hence, the presence of *E. coli* in foods or water became accepted as an indicator of fecal contamination or unsanitary processing and the possible presence of intestinal pathogens (Feng *et al*., 2002). Infants and young children are particularly vulnerable to foodborne illnesses because their immune systems are not developed enough to fight diseases. In fact, 800,000 illnesses affect children under the age of 10 in the U.S. each year (CFSAN, 2005). Globally, diarrheal diseases, including cholera, shigellosis and rotavirus, kill about 1.8 million children under the age of 5 each year (Bryce *et al*., 2005). In 1993 a multistate outbreak of *E. coli* O157:H7 resulted in 501 cases including 151 hospitalizations, 45 cases of hemolytic uremic syndrome and 3 deaths (Bell *et al*., 1994).

Opened jars of baby food or freshly made baby foods may become contaminated with fecal material from kitchen and other area surfaces and from those changing children diapers but who do not practice adequate personal hygiene. Hands can pick up bacteria, from diapers containing feces and urine and spread them. Indeed, some studies observed that 58% of people caring for children did not wash their hands with soap and 32% did not wash their hands after changing a dirty diaper (Curtis *et al*., 2003; CFSAN, 2005). Diaper changing may take place in a number of places including in living rooms and kitchens. Evidence of fecal contamination was found in living room and in kitchen surface samples (Curtis *et al*., 2003). Fecal coliform contamination of environmental surfaces (inanimate objects, toy balls, etc.) and hands in day-care centers were also noted (Van *et al*., 1991). Baby foods from opened jars or freshly made ones may be frozen for later use. The Center for Food and Applied Nutrition recommendation for safe frozen storage of baby foods is from 1-8 months depending on the baby food product (CFSAN, 2005).

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Freezing, frozen storage and thawing are known to both damage and destroy a portion of a bacterial population such as *E. coli* (Uyttendaele *et al*., 2002; Dykes, 2000). Freezing brings about a decrease in the colony forming ability of bacteria in non-selective media due to lethal damage. Water removal as ice, solute concentration, intracellular ice crystals formation during freezing and recrystalization during frozen storage and thawing are considered to be major physical and chemical factors responsible for lethal and non-lethal damage to bacterial cells such as *E. coli* (Souzu, 1989). The cell membrane can also be destroyed by the spearing action of ice crystal (Ray and Speck, 1973). Non-lethal damage from freezing, frozen storage and thawing increases the sensitivity of certain bacteria, including *E. coli*, to surface acting compounds. For example, *E. coli* damaged by freezing becomes sensitive to selective agents such as deoxycholate or lauryl sulphate used in selective media. Presumably, the impaired cell membrane allows these compounds into the cell leading to an inhibitory action. Non-lethally damaged bacterial cells of *E. coli* may lose their ability to form colonies in selective media (Raccach *et al*., 2002).

The objective of this study was to evaluate the effects of freezing and frozen storage (-18°C) on *E. coli* inoculated in baby foods.

MATERIALS AND METHODS

Experimental foods: The commercial baby foods Banana Tapioca (BT), Creamed Corn (CC) and Strained Peas (SP) were used in this study.

Culture: *Escherichia coli* K12 (ATCC 25404) was maintained (4°C) as a stock culture on slants of Tryptic Soy Agar (TSA, Difco Detroit, Michigan, USA). Every 2 months, the stock culture was checked for purity and subsequently subcultured on TSA slants.

Preparation of the experimental culture: One loopful of the stock culture of *E. coli* was inoculated into 5 mL of Tryptic Soy Broth (TSB, pH 6.78, Difco, Detroit, Michigan, USA) and incubated (24 h, 35°C). Three daily (every 20-24 h) successive transfers in sterile TSB were undertaken to rejuvenate the culture. Each subculture was incubated (24 h, 35°C) and the culture from the third transfer was used to inoculate (1% v/v) sterile TSB. The inoculated TSB was incubated (24 h, 35°C) to obtain the working culture.

Inoculation of the experimental foods: Each baby food was inoculated with the working culture (0.45% v/w) to obtain about 7 $\log_{10}$ cfu g^{-1}. Thirty, 10 g aliquots, from each baby food, were aseptically dispensed into sterile covered aluminum containers. All the inoculated experimental food samples were stored (-18°C) for up to 26 days in the freezer compartment of a laboratory refrigerator. After being placed in the freezer, the inoculated samples solidified within about 1.5 h.

Enumeration media and procedure: The non-selective medium, Plate Count Agar (PCA, Difco, Detroit Michigan, USA) and the selective medium, Violet Red Bile Agar (VRBA, Difco, Detroit Michigan, USA), were used to enumerate *E. coli*.

One container (10 g) from each of the inoculated baby foods was sampled and enumerated, in each of the enumeration media, before and daily (every 24 h) during the frozen storage (-18°C) period of 26 days. Ten gram of each thawed (25-27°C) baby food sample was diluted with 90 mL sterile peptone (0.1% w/v) water in a sterile Erlenmeyer flask and mixed to homogeneity using a Vortex. Appropriate decimal dilutions of the inoculated baby food sample in sterile peptone (0.1% w/v) water were pour plated, in duplicates, with each PCA and VRBA. The media were incubated (24 h, 35°C) and enumerated. Non inoculated samples from each baby food did not show any growth in PCA.

Fitting mathematical models to the experimental data: Mathematical equations were fitted to the experimental data using DataFit (V.2.1, A Soft Answer P.O. Box 1743 Macquarie Centre NSW 2113 Australia) a generalized function fitting and data modeling program. The Nelder and Mead simplex fitting method was used (Nelder and Mead, 1965). For fitting purposes, the experimental data from each PCA and VRBA was used in the form of cfu g^{-1}. The best mathematical equation was selected as one having the highest correlation coefficient (R), the lowest standard deviation and the lowest chi-square (between the model and data). Solving a mathematical equation for a particular day of frozen storage provided the model population (cfu g^{-1}) for that day. When needed, the model population (cfu g^{-1}) was transformed and expressed as log cfu g^{-1}.

Definitions and estimates of the frozen-thawed populations of *E. coli*: The definitions with some modifications are based on previously published work (Raccach *et al*., 2002; Sage and Ingham, 1998). The use of the term damage indicates in this study non-lethal damage. All the calculations were done using populations of *E. coli* (cfu g^{-1}) obtained from the mathematical model equations.

Viable population: The viable Population (Pv) of *E. coli* developed colonies in the non-selective medium PCA. This population is comprised of undamaged and damaged cells of *E. coli* (definitions follow). The size of the viable population was calculated as follows:

Viable population = (Model population from PCA after a frozen storage period t)

Undamaged population: The undamaged population (Pud) is that portion of the viable population of *E. coli* that was able to form colonies in the selective medium VRBA. The size of the undamaged population was calculated as follows:

Undamaged population = (model population from VRBA after a frozen storage period t)

Damaged population: The damaged population (Pda) is that portion of the viable population of *E. coli* that was unable to form colonies on the selective medium VRBA. This is a non-lethal damage. The difference between the numbers of colonies formed in PCA and in VRBA after a frozen storage period t has been used as a means to determine the degree to which a microbial population is damaged (Raccach *et al*., 2002; Sage and Ingham, 1998; Ray and Speck, 1973). The size of the damaged population was calculated as follows:

$$\text{Damaged population} = \begin{pmatrix}\text{Model population from PCA}\\ \text{after a frozen storage period t}\end{pmatrix} - \begin{pmatrix}\text{Model population from VRBA}\\ \text{after a frozen storage period t}\end{pmatrix}$$

Statistical analyses: The results of this study are the average of 2 independent experimental trials.

Analyses of variance and paired t test were done where applicable. A value of p = 0.05 denotes statistical significance to the experimental observation.

Analysis of variance: Split plot analyses of variance (Steel and Torrie, 1980) were done to statistically evaluate the effects of freezing, baby food products and culture media on *E. coli*.

Paired t-test: The t test was used to statistically evaluate paired experimental observations (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The mathematical model equations: The mathematical model equations for both the viable (Pv) and undamaged (Pud) populations of *E. coli* in each of the baby foods Banana Tapioca (BT), Creamed Corn (CC) and Strained Peas (SP) are presented in Table 1. The values of the correlation coefficient (R) between the experimental data and the model equations were between 0.9819 and 0.9999. The values of the chi square and the standard deviation associated, for example, with the model equation for the viable population in BT were 8.2085×10^{-9} and 7.3611×10^{-5}, respectively and those associated with the model equation for the undamaged population in CC were 2.4545×10^{-2} and 12295, respectively.

Table 1: The mathematical model equations of the viable (Pv) and undamaged (Pud) populations (cfu g^{-1}) of *E. coli* in baby foods

Baby food	Population	R
Banana tapioca	$Pv = (2.83*10^{-8}+(6.18*10^{-8}*t))^{-1}$	0.9937
	$Pud = \exp(15.026+(-0.1488*t)+(1.7352*\exp(-t)))$	0.9992
Creamed corn	$Pv = (6.26*10^{-8}+(6.45*10^{-7}*t))^{-1}$	0.9997
	$Pud = (1.76*10^{-7}+(4.59*10^{-6}*t))^{-1}$	0.9999
Strained peas	$Pv = (1495.4+(-269.82*(t)^{1/2})+(1624.8*\exp(-t)))^{2}$	0.9819
	$Pud = (1.41*10^{-7}+(9.16*10^{-8}*t)+(4.48*10^{-8}*t^{3}))^{-1}$	0.9996

t is time (days) of frozen storage; R is the Correlation coefficient; exp is the exponent

Each model equation enables the prediction of the level of the respective population of *E. coli* within the frozen storage (-18°C) period of 26 days. For example, after 2.5 and 24.5 days of frozen storage the viable population in BT and the undamaged population in SP would be about 6.7 and 3.2 log cfu g^{-1}, respectively.

The model equations make it possible to predict the time it would take, in frozen storage, to reduce a population to a certain level. For example, to reduce a population to a level of 5.0 log cfu g^{-1}, it would take about 15.4 and 2.5 days of frozen storage for the viable population in CC and for the undamaged population in SP, respectively.

The model equations may also be used to calculate the damaged population (Pda) of *E. coli* at any time during frozen storage by subtracting the model undamaged population (Pud) from the model viable population (Pv). For example, after 10 days of frozen storage the level of the damaged population would be about 5.9, 5.1 and 5.6 log cfu g^{-1} in BT, CC and SP, respectively.

Effect of freezing and frozen storage on the populations of *E. coli*: The effect of freezing and frozen storage (-18°C) on the model viable (Pv) and undamaged (Pud) populations of *E. coli* in BT, CC and SP are shown in Fig. 1 and 2, respectively. Before freezing, there was no statistical difference (p = 0.62) between the viable population and the undamaged population. Similar results were obtained with *E. coli* O157:H7 (Raccach *et al*., 2002). After 1 day of frozen storage, the viable and undamaged

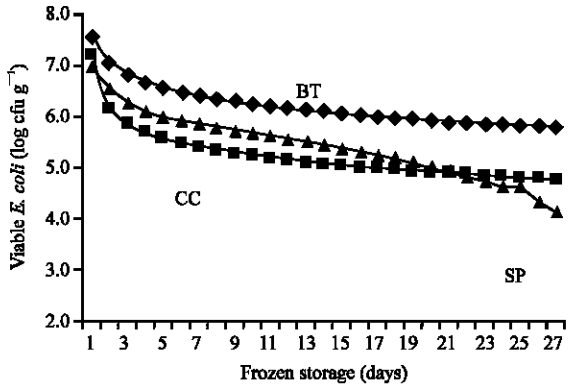

Fig. 1: The model viable population of *E. coli* (Pv) after freezing and frozen storage (-18°C). BT is Banana Tapioca; CC is Creamed Corn and SP is Strained Peas

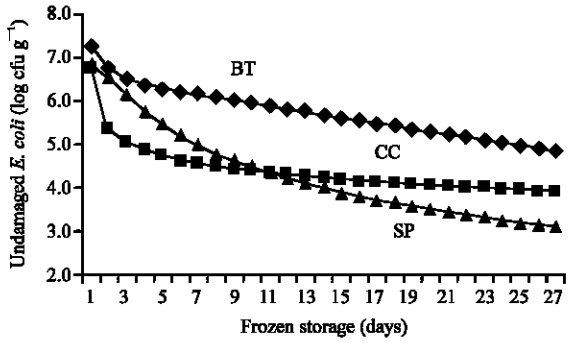

Fig. 2: The model undamaged population of *E. coli* (Pud) after freezing and frozen storage (-18°C). BT is Banana Tapioca; CC is Creamed Corn and SP is Strained Peas

populations of *E. coli* in the three baby foods were reduced by 65.9-91.2 and 63.0-98.7%, respectively. Both the viable and undamaged populations in the three baby foods declined as the frozen storage period was prolonged (Fig. 1 and 2).

Viable population: The viable population declined after 26 days of frozen storage by about 2.8 (SP), 2.4 (CC) and 1.7 (BT) log cfu g^{-1} (Fig. 1). This is in agreement with other studies (Uyttendaele *et al*., 2002; Dykes, 2000). This may indicate the potential for survival of virulent strains of *E. coli* and other intestinal pathogens in baby foods stored frozen. The analysis of variance for the effect of freezing and frozen storage on the viable population in the three baby foods (Table 2), indicated that the destructive effect of freezing was statistically significant (p = 0.013). Neither the effect of baby foods (p = 0.36) nor the interaction between freezing and baby foods (p = 0.71)

Table 2: Analysis of variance: The effect of freezing and frozen storage (-18°C) on the viable population of *E. coli* in baby foods

Source of variation	DF*	SS	Mean square	F	p
Blocks	1.00	8928.03	8928.03		
Foods, A	2.00	28714.73	14357.37	1.75	0.36
Error (a)	2.00	16398.27	8199.14		
Freezing, B	4.00	180187.75	45046.94	4.99	0.013**
Interaction, AB	8.00	48365.74	6045.72	0.67	0.71
Error (b)	12.00	108241.52	9020.13		
Total	29.00	390836.05			

*DF = Degree of Freedom; SS = Sum of Squares; F = F value and p=calculated probability of statistical significance; A = Banana Tapioca, Creamed Corn and Strained Peas, **Statistically significant

Table 3: Analysis of variance: The effect of freezing and frozen storage (-18°C) on the undamaged population of *E. coli* in baby foods

Source of variation	DF*	SS	Mean square	F	p
Blocks	1.00	1270.44	1270.44		
Foods, A	2.00	8756.64	4378.32	0.95	0.51
Error(a)	2.00	9223.56	4611.78		
Freezing, B	4.00	49210.63	12302.66	24.26	0.000011**
Interaction, AB	8.00	15869.54	1983.69	3.91	0.017**
Error(b)	12.00	6084.45	507.04		
Total	29.00	90415.26			

*DF = Degree of Freedom; SS = Sum of Squares; F = F value and p=calculated probability of statistical significance; A = Banana Tapioca, Creamed Corn and Strained Peas, **Statistically significant

were as significant as freezing. The highest and lowest levels of survivors of the viable population were in BT and CC (up to 21 days), respectively (Fig. 1). For example, after 7 days of frozen storage, the difference between the viable population in BT and that in CC was substantial (p=0.047). After 21 days of frozen storage the number of viable survivors of *E. coli* in SP started to decline slightly (p=0.49) more than those in CC.

Undamaged population: The undamaged population declined after 26 days of frozen storage by about 3.8 (SP), 2.9 (CC) and 2.5 (BT) log cfu g^{-1} (Fig. 2). The analysis of variance for the effect of freezing, frozen storage on the undamaged population in the three baby foods, revealed that both freezing (p=0.000011) and the interaction between freezing and baby foods (p=0.017) were statistically significant (Table 3). This may have a practical implication since either the presence or the level of fecal coliform are routinely determined using selective media. After 11 days of frozen storage (Fig. 2), the number of survivors of the undamaged population of *E. coli* in SP slightly started to decline more than that in CC (p = 0.69).

Since the undamaged population is part of the viable population, a decrease in the undamaged population may indicate a decrease in the viable population. A decrease in the viable population suggests a larger lethal effect of freezing and frozen storage. BT may have provided more cryoprotection for both the viable and undamaged

populations than either CC or SP (Fig. 1 and 2). This may be due to the level of non-fiber complex carbohydrates of 78.4% (USDA, 2006) in BT as compared to CC and SP (60.6 and 32.7%, respectively). Cryoprotective agents such as complex carbohydrates have the ability to reduce the damage to cells from freezing (Breierova, 1998). For example, a solution of 12% (w/w) starch contributed to an 86-93% survival rate of erythrocytes (Sputtek *et al*., 1992).

Throughout the frozen storage period the level of the undamaged population, in the tested baby foods, was constantly lower than that of the viable population. The undamaged population of *E. coli* declined by 0.5-1.0 log cfu g^{-1} more than the viable population. Raccach *et al*. (2002) found that after 7 days of frozen storage (-18°C) the undamaged population of *E. coli* O157: H7 was reduced by 97%. Freezing may have increased the sensitivity of the cells of *E. coli* to selective agents in VRBA (Raccach *et al*., 2002; Souzu, 1989). A split plot analysis of variance for every baby food (examining the effect of freezing and culture media) indicated that freezing was a significant factor reducing the population of *E. coli* (the F ratios and values of p were 4.28 and 0.038; 11.8 and 0.00197 and 3.95 and 0.047 in BT, CC and SP, respectively). Each of these analyses of variance also indicated no statistical significant difference, in every baby food, between the numbers of colonies of *E. coli* in VRBA as compared to PCA. The F ratios and values of p were 0.84 and 0.53; 1.59 and 0.43 and 1.10 and 0.48 in BT, CC and SP, respectively.

Damaged population: A damaged population of *E. coli* was noted from the difference between the viable population in PCA and the undamaged population in VRBA. As stated above, no statistical significant difference was found between the numbers of colonies of *E. coli* in VRBA as compared to that in PCA. The highest levels of damaged *E. coli* were 5.6×10^{6}, 1.2×10^{6} and 5.5×10^{5} cfu g^{-1} in BT, CC and SP, respectively. The damaged population of *E. coli* progressively decreased as the frozen storage period was extended. For example, after 26 days of storage the level of damaged cells of *E. coli*, in all baby foods, decreased up to >40 times (5.4×10^{5}, 5.1×10^{4} and 1.3×10^{4} cfu g^{-1} in BT, CC and SP, respectively) compared to their respective highest levels. This is in agreement with Raccach *et al*. (2002) who found that after 7 days of frozen storage (-18°C) the damaged population of *E. coli* O157: H7 was reduced by 45%.

CONCLUSION

After 26 days of frozen storage the viable, undamaged and damaged populations of *E. coli*, in the baby foods used in this study, were in the range of 4.1-5.8 log cfu g^{-1}. If baby foods become contaminated with fecal material freezing would not destroy all contaminants. Virulent strains of *E. coli* that survive freezing may have adverse effects on babies, infants and young children because of their high vulnerability to illnesses.

REFERENCES

Armstrong, G.L., J. Hollingsworth and J.G. Morris, 1996. Emerging food borne pathogens: O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiol. Rev., 18: 29-51.

Bell, B.P., M. Goldoft, P.M. Griffin, M.A. Davis and D.C. Gordon *et al*., 1994. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. J. Am. Med. Assoc., 272: 1349-1353.

Breierova, E., 1998. Yeast Exoglycoproteins Produced Under NaCl-Stress Conditions as Efficient Cryoprotective Agents. Biol. Proc. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=140111.

Bryce, J.C. Boschi-Pinto, K. Shibuya and R.E. Black, 2005. WHO Child Health Epidemiology Reference Group. WHO estimates of the causes of death in children. Lancet, 365: 1147-1152.

CFSAN (Center for Food Safety and Applied Nutrition), 2005. Food Safety for Moms-To-Be Once Baby Arrives. US Food and Drug Administration. http://www.cfsan. fda.gov/~pregnant/once.html.

Clarke, S.C., 2001. Diarrhoeagenic *Escherichia coli* An emerging problem? Diagnostic Microbiol. Inf. Dis., 41: 93-98.

Curtis, V., A. Biran, K. Deverell, C. Hughes, K. Bellamy and B. Drasar, 2003. Hygiene in the home: relating bugs and behaviour. Soc. Sci. Med., 57: 657-672.

Dykes, G. A., 2000. The effect of freezing on the survival of *Escherichia coli* O157:H7 on beef trimmings. Food Res. Int., 33: 387-392.

Feng, P., S.D. Weagant and M.A. Grant, 2002. Enumeration of *Escherichia coli* and the Coliform Bacteria Bacteriological Analytical Manual on line, Chapter 4. http://www.cfsan.fda.gov/~ebam/bam-toc.html

Kuntz, T.B. and S.T. Kuntz, 1999. Enterhaemorrhagic *E. coli* infection. Primary Care Update Ob/Gyns., 6: 192-195.

Nelder, J. and R. Mead, 1965. The downhill simplex method. Compu. J., 7: 308-310.

Raccach, M., T. Bamiro, J. Clinch, G. Combs, A. Gierczynski and R. Karam, 2002. Frozen storage of *Escherichia coli* O157: H7 in vegetable broth. Food Control, 13: 381-385.

Ray, B. and M.L. Speck, 1973. Freeze-injury to bacteria. CRC Critical Rev. Clin. Lab. Sci., 4: 161-213.

Sage, J.R. and S.C. Ingham, 1998. Survival of Escherichia coli O157: H7 after freezing and thawing in ground beef patties. J. Food Prot., 61: 1181-1183.

Souzo, H., 1989. Changes in chemical structure and function in *Escherichia. coli* cell membranes caused by freeze-thawing. I. Change of lipid state in bilayer vesicles and in the original fragments depending on rate of freezing. Biochim. Biophys. Acta., 978: 105-111.

Sputtek, A., C. Bacher, R. Langer, W. Kron, H.A. Henrich and G. Rau, 1992. Cryopreservation of Human Erythrocytes with Hydroxyethyl Starch (HES)-Part 2: Analysis of survival. Infusionstherapie and Transfusionsmedizin, 19: 276-282. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=1284211&query_hl=6&itool=pubmed_docsum.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and procedures of statistics. 2nd Edn. McGraw-Hill Company, New York USA.

USDA, 2006. National Nutrient Database for Standard Reference. Nutrient data laboratory. Agriculture Research Service. http://www.nal.usda.gov/fnic/foodcomp/search/index.html

Uyttendaele, M., S. Vankeirsbilck and J. Debevere, 2002. Recovery of heat-stressed *E. coli* O157:H7 from ground beef and survival of *E. coli* O157:H7 in refrigerated and frozen ground beef and in fermented sausage kept at 7 and 22°C. Food Microbiol., 18: 511-519.

Van, R., C.C. Wun, A.L. Morrow and L.K. Pickering, 1991. The effect of diaper type and overclothing on fecal contamination in day-care centers. J. Am. Med. Assoc., 265: 1840-1844.