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# Outbreak of *E. coli* 0157:H7 Infections Associated With Ready-To Eat Cashew Nuts in a Nigerian University Community

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**ABSTRACT:** Microbiota of retail ready-to-eat cashew nuts was investigated due to outbreak of *E. coli* 0157:H7 infections during 2006 and 2007 respectively. Moisture content: 3.7 -4.3 and 3.2 - 5.4 respectively. Mesophillic bacteria: 4 - 19 x $10^4$cfu/g; *Staphylococcus aureus*: 2 - 4 x $10^4$ cfu/g; Coliform count: 2 - 3.0 x $10^4$ cfu/g; Fungal count: 14 - 25 x $10^4$ cfu/g were recorded. *Aspergillus flavus, A. parasiticus, A. niger , A. terreus, Rhizopus* were predominant. Aflatoxins determined using TLC Scanner 3 ranged from 1.4 to 6.0 ng/g. Serologic test confirmed presence of *E. coli* 0157:H7. Sanitary measures should be implemented.

**KEY WORDS:** Aflatoxin, cashew nuts, diarrhoea, *E. coli* serogroup 0157:H7, microbiota, TLC Scanner 3.

## INTRODUCTION

Food-borne illnesses in human beings due to microbial origin are well documented world-wide (Hazariwala et al., 2002; Johnston et al., 2005; Laine et al., 2005; Hussein and Sakuma, 2005; Vanselow et al., 2005). The large number of cases of human illness caused by Shiga toxin- producing E coli (STEC) world-wide has raised safety concerns for foods. These human illnesses include diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome and thrombotic thromocytopenic purpura. Severe cases end with chronic renal failure, chronic nervous system deficiencies and death (Hussein and Sakuma, 2005; Frenzen et al., 2005; Laine et al., 2005; Vanselow et al., 2005).

Food-borne illness imposes a substantial economic and quality of life burden on society by way of acute morbidity and chronic sequela (Duff et al., 2003. Frenzen et al., 2005). Food safety presents a double moral hazard problem in that it affects both consumers and producers (Elbasha and Riggs, 2003. The Centers for Disease Control and Prevention (CDC) estimated 73,000 illness caused by *E. coli* (0157STEC), 2,000 hospitalization, 60 deaths, $ 370 million premature deaths, $ 30 million for medical care, $ 5 million in lost productivity and $6.2 million for a patient who died from haemolytic uremic syndrome (Frenzen et al., 2005). There were 44451 sporadic case of illness attributable to 8 enteric pathogens *Campylobacter*, *Salmonella*, verotoxin – producing *Escherichia coli*, *Yersinia*, *Shigella*, Hepatitis A, *Listeria* and *Clostridium botulinum* in Ontario (Lee and Middleton, 2003). In Japan, outbreak of *E coli* 0157:H7 in elementary school children cost about 82,686, 000 yen (Abe et al., 2002).

A group of researchers believe that the distribution of monthly and seasonal patterns of food-borne illness outbreaks strongly indicate the outbreaks may be associated with climatic conditions, frequencies of national holidays and vacation seasons (Lee et al., 2001). However, there is a dearth of information on food-borne illness especially on cashew nuts in Nigeria. Cashew nut is an excellent substrate for microorganisms with fat content of 41.13%; nitrogenous matter of 9.7%; starch 5.9% protein

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14;43%; ash 2.58% and fibre 1.27% (Alice, 1994). Therefore, due to the high nutritional values of cashew nuts, they are subject to microbial contamination although serious bacterial infection on cashew nuts has rarely been reported. Plant pathological surveys conducted in the past ten years have shown that over 10% of the annual crop is unfit for human consumption as a result of microbial biodeterioration and therefore cannot be exported with an accompanying loss of revenue. Both bacteria and fungi are responsible for these losses. Over 60 species of fungi have been identified and many others are now included (Freire et al., 1999; Freire and Bargail, 2001; Adebajo and Diyaolu, 2003). Growth of bacteria and yeast may take place but occurs in small numbers mostly if the environmental conditions are damp and when equipment and workers contribute to increase in the level of contamination (Muniz, 2004).

During the the second semester examination in 2006 at the University of Agriculture, Abeokuta, Nigeria, Cashew nuts were served as snacks to University lecturers and most of the lecturers came down with stomach upset, nausea and diarrhoea. This led to investigation on the source of the snacks during the dry season of 2006 and the rainy season of 2007.

The objectives of this study were to ascertain the sources of the hazard by determining the bacteriological quality of ready – to – eat cashew nuts, isolate and characterize contaminating fungi, quantify levels of aflatoxin and educate factory food workers on how to decrease cross contamination by hand washing with a mild soap and water.

## MATERIALS AND METHODS

### Source and collection of samples:

Twelve samples of cashew nuts made up of whole, small white piece and split nuts that were both salted and unsalted were purchased from the factory situated within the University of Agriculture, Abeokuta, Nigeria. Samples were collected randomly during the dry season of 2006 and the raining season of 2007. Samples of cashew nuts were transported in sterile containers to the International Institute of Tropical Agriculture, Ibadan, Nigeria. All analyses were carried out in the pathology laboratory of the Institute.

Moisture content and pH determination: Ten grammes of each cashew nut sample was weighed into a sterile mortar and crushed. The suspension of the pulversised material (1:2 (w/v) in distilled water was obtained and pH determined using pH meter (Kent EIL 7020). Moisture content was determined by filling a rapid moisture tester (Hand Moisture tester, Dickey – John) with cashew nuts.

Bacteriology: Total and differential counts. Ten gram of each sample of cashew nut was grinded in an electrical blender for 3 minutes and 1g weighed out into a test-tube previously autoclaved at 121$^{o}$C for 15min. Ten milliliters of sterile distilled water was poured into the first test-tube and vortexed for 1min, thereafter 1ml of the suspension was transferred to subsequent test tubes each containing 9 ml and serial dilutions up to $10^{-7}$ dilution were done to determine total viable counts. 1 ml of each of $10^{-4}$ and $10^{-6}$ dilutions were plated on nutrient agar plates in 5 replicates. The plates were incubated at 37$^{o}$c for 24 hours. The same procedure was repeated for *Staphylococcus aureus* count, enterobacteriaceae count on mannitol salt agar and MacConkey agar respectively.

Identification of bacterial isolates: Isolates on mannitol salt agar and Mac Conkey agar were identified based on established conventional cultural, morphological and biochemical characterization (Encinas et al., 1996).

Serological test: After the identification of colonies by macroscopic, microscopic and biochemical means, pink colonies on MacConkey agar plates which tested positive to indole, were serotyped to determine the serogroup and pathogenicity using *E. coli* O157 latex test(product DR620, Oxoid, Basingstoke, UK).

A selective and differential medium sorbitol MacConkey agar for the detection of *E. coli* 0157 was prepared according to instruction on the kit. At a temperature of 45$^{o}$C, the molten agar was dispensed into petri-dishes and allowed to set. The organism to be tested was inoculated on the petri-dishes and incubated at 37$^{o}$C for 24hrs. Colourless colonies was a preliminary positive test that *E. coli* 0157 was present.

A confirmatory test was done using a latex agglutination test (Product DR 620, Oxoid, UK). A drop of the latex was dispensed on the circles on the reaction card. Drops of normal saline were added to the circle ensuring that the latex and the saline did not mix at this stage using a sterile loop. A portion of the colony to be tested was picked and carefully emulsified in the saline drop. The latex was mixed with the saline containing test organism and rocked in a circular motion for 1 min and reaction observed for a positive or negative test.

### Total fungi count and identification

Serial dilutions of $10^{-4}$ and $10^{-6}$ were plated on saboraud dextrose agar and incubated at 28 $^{o}$C for 5 days. All

colonies were counted and expressed in cfu/g of the sample.

Identification of fungal colonies isolated fungal species were observed macro and microscopically. Fungal isolates were stained with cotton blue lactophenol and visualized under X40. Identification was done with reference to International standard used by Klich (2003) for identifying moulds.

**Aflatoxin extraction and quantification**

Ten grammes of cashew nuts were milled with 70% methanol using electric blender for 3 min at high speed. The ratio of sample to extracting solvent was 1:5. The milled suspension was filtered using Whatman no.1 filter paper (Whatman International Ltd, Maidstone, UK). To each volume of extract was added 100ml of distilled water in a separating funnel containing a bed of anhydrous sodium sulphate and 25ml of dichloromethane. This was shaken vigorously for 2 min and the lower layer collected. The extraction was done 2 times and pooled together for evaporation to dryness in the absence of light. The dry extract was reconstituted by adding 1 ml of dichloromethane and spotted on thin layer chromatography (TLC) plate 20cm by 20cm using a 4 ul glass capillary micropipette. The plates were developed in 20 ml of the developing solvent ( diethyl- ether- methanol water (96:3:1).

The detection wavelength for excitation and emission were 366 and 400nm. The developed plates were viewed under UV light at 366nm. The plates were positioned in the scanner with the aid of internal illumination. TLC scanner 3 which is the most advanced workstation for densitometric evaluation and WinCATS (CAMAG); a novel integrated software concept which incorporated all steps of TLC were used. After scanning, the peak values along both X – axis and Y – axis were determined for unknown and known samples. The spectrum pathway for all aflatoxins suspected followed the same pathway as that of the standard.

**Experimental design and statistical analysis**

A factorial design involving a 2 x 2 x 3 was employed. All assays were carried out in five replicates and data analysed using analysis of variance (ANOVA), Duncan multiple range test and confirmed with least significant difference test using SAS windows software.

## RESULTS

Moisture content and pH values of cashew nuts

Table 1 showed the moisture content and pH values of cashew nuts during both dry and wet seasons. Values of moisture content were low indicating that the roasting and packaging processes were adequate although there was a slight difference between dry and wet seasons which was significantly different at $P< 0.05$ (whole salted 4.3 and 5.3 respectively). Salted and unsalted cashew nuts in both seasons were below 5.8. Salts and sugars in solution have been used to reduce moisture content apart from flavouring (Falade et al., 2003). Results showed significant difference between the mc of salted and unsalted cashew nuts.

Microbial counts in cashew nuts

Although cashew nut is an excellent substrate for microorganisms, occurrence of serious bacterial infection has rarely been reported. Table 2 gave counts of mesophilic bacteria, enterobacteriaceae, *S. aureus* as low as between 1 and 14 x $10^4$ cfu/g. Mesophilic bactera counts on roasted and sated Brazillian nuts ranged from 5.3 x $10^3$ and 1.2 x $10^4$ cfu//g (Francisco et al, 2002). These values are in agreement with the findings in this study.

*Staphylococcus aureus* contamination has been previously reported in cashew nut outside Nigeria that they occur also in low numbers (King and Jones, 2001). The range of counts of *S. aureus* was between 2 and 7 x $10^4$ cfu/g. This finding is shown in Tables 2a and 2b. Enterobacteriaceae counts were low as recorded in Tables 2a and 2b between 1 and 3 x $10^4$ cfu/g.

**Table 1**

Moisture content(mc) and pH levels of cashew nuts obtained during dry and raining seasons of 2006 – 2007.

| Cashew nuts | Dry Season | | Raining Season | |
|---|---|---|---|---|
| **Type** | M.C (%) | pH | M.C (%) | pH |
| **Whole** | | | | |
| salted | 4.3 | 5.1 | 5.3 | 5.3 |
| Unsalted | 4.3 | 5.1 | 5.4 | 5.1 |
| **Small white piece** | | | | |
| salted | 4.0 | 5.3 | 3.2 | 5.2 |
| Unsalted | 3.9 | 5.0 | 5.1 | 5.5 |
| **Split** | | | | |
| salted | 3. | 5.1 | 4.2 | 5.4 |
| Unsalted | 3.9 | 5.0 | 4.9 | 5.4 |

**Confirmation of E. coli 0157:H7**

The outbreak of *E. coli* 0157:H7 in this study was confirmed apart from stomach upset, nausea, diarrhoea from the lecturers who ate the cashew as snacks served during the second semester examination of 2006,

biochemical and serologic tests using slide agglutination test (product DR620, Oxoid, Basingstoke, UK) confirmed the presence of *E. coli* 0157:H7 in ready-to-eat cashew nuts.

Fungal counts, identification and aflatoxins concentration in cashew nuts

Fungal counts were the highest counts (Tables 2a and 2b) ranging from 1 to 14 x $10^4$ cfu/g. Organisms isolated and characterized macro and microscopically using the methods of Klich (2002) were *Aspergillus niger, A. flavus, A. restrictus, Penicillium spp. Rhizopus spp.*

Table 3 showed the results of aflatoxins quantified in the cashew nuts using Thin Layer Chromatography (TLC) scanner 3 which is the most advanced workstation for densitometric evaluation and WinCATS (CAMAG)- a novel integrated software concept which incorporates all steps of TLC to control all the functions of scanner 3. Positive samples during the dry season range between 1.34 to 5.84 ng/g which was above the level (0.35ng/g) recorded by Leszczynska et al., (2000).

**Table 2a** Microbial counts $10^4$cfu/g) of cashew nuts (cfu/g) during dry season in the year 2006.

| Media | whole nuts | | split nuts | | small white piece | |
|---|---|---|---|---|---|---|
| | **Unsalted** | **salted** | **Unsalted** | **salted** | **Unsalted** | **salted** |
| Nutrient Agar | 4 | 3 | 3 | 2 | 1 | - |
| MacConkey Agar | 2 | - | 1 | 3 | 1 | - |
| Mannitol salt Agar | 2 | - | 2 | 1 | - | 2 |
| Saboraud Dextrose Agar | 14 | 2 | 12 | 1 | 10 | 6 |

**Table 2b** Microbial counts $10^4$cfu/g) of cashew nuts (cfu/g) during dry season in the year 2006.

| Media | whole nuts | | split nuts | | small white piece | |
|---|---|---|---|---|---|---|
| | **Unsalted** | **salted** | **Unsalted** | **salted** | **Unsalted** | **salted** |
| Nutrient Agar | 5 | 10 | 19 | - | 12 | 3 |
| MacConkey Agar | - | - | - | 2 | 1 | 1 |
| Mannitol salt Agar | - | 1 | 3 | 1 | 7 | 2 |
| Saboraud Dextrose Agar | 9 | 13 | 19 | - | 25 | 21 |

**Table 3**

Total afflatoxin levels (ng/g) in cashew nuts during the dry season of 2006.

| Cashew type | *B1 | B2 | G1 | G2 | Total |
|---|---|---|---|---|---|
| USLOP | nd | 1.34 | nd | nd | 1.34 |
| SSWP | 1.62 | 0.92 | 2.0 | nd | 2.54 |
| SW | nd | 5.84 | nd | nd | 5.84 |
| UW | nd | 2.41 | 1.31 | nd | 3.72 |
| SS | nd | nd | nd | nd | 0 |
| US | nd | nd | nd | nd | 0 |

Average result of 5 replicates

USWP = Unsalted small white piece;
SSWP = Salted small white piece
SW = Salted whole
UW = Unsalted whole
SS = Salted split
US = Unsalted split
ND = Not detectable

## DISCUSSION

Moisture content (mc) and pH values are critical factors that determine microbial contamination of foods (Henderson, 1985; Oluwafemi and Adegoke, 2004). Values of moisture content were low indicating that the roasting and packaging processes were adequate although there was a slight difference between dry and wet seasons which was significantly different at P< 0.05 (whole salted 4.3 and 5.3 respectively). This is because cashew nuts are colloids and hygroscopic when placed in environment of high relative humidity picks up moisture until equilibrium is reached (Oluwafemi and Adegoke, 2004). The safe moisture content for storage of nuts is 5.8 (Henderson, 1985) and both salted and unsalted cashew nuts in both seasons were below 5.8. Salts and sugars in solution have been used to reduce moisture content apart from flavouring (Falade et al., 2003). Results showed significant difference between the mc of salted and unsalted cashew nuts. The reason might be probably due to osmosis.

Although cashew nut is an excellent substrate for microorganisms, occurrence of serious bacterial infection has rarely been reported. Studies conducted in South Africa revealed light contamination of processed

and non-processed cashew nuts. Mesophilic bactera counts on roasted and salted Brazillian nuts ranged from 5.3 x $10^3$ and 1.2 x $10^4$ cfu//g (Francisco et al, 2002). These values are in agreement with the findings in this study. The low numbers of bacteria might be due to injured or stressed condition and that recovery steps should be built into the protocol (Duffy et al., 2004), otherwise a false negative result will be recorded. The low numbers of bacteria recorded in cashew nuts could also be attributed to natural inhibitors of bacterial growth which interfere with isolation and detection (Muroi and Kubo 1993, Kubo et al, 2003). The Scientists were of the opinion that the presence of anacardic acids and phenolic compounds present in cashew nut oil have contributed to some inhibitory action against gram – positive bacteria, yeast and fungi. Anacardic acids with distinct lateral chains have been shown to be effective against the growth of *Staphylococcus aureus, Propionibacteria, spp, Streptococcus mutans* and *Brevibacterium ammoniages* (Torquato, 2004).

*Staphylococcus aureus* contamination has been previously reported in cashew nut outside Nigeria that they occur also in low numbers (King and Jones, 2001). This finding is in agreement with results obtained in Tables 2a and 2b on *S. aureus* count. Processing conditions such as heating (roasting) and salting would have resulted in reducing *S. aureus* count in addition to the presence of anacardic acid. According to Shebuski and Vilhelmsson (2000), *S. aureus* is the most osmotolerant food-borne pathogen and that outbreaks of staphylococcal food poisoning are often linked to foods of reduced water activity values. The presence of catalase positive *S. aureus* could be a causative agent of food poisoning since it produces various super-antigenic exotoxins which have a great public health significance (Yoon and Kim, 2003). In humans, *S. aureus* can lead to septicaemia (Hazariwala et al. 2002), although no case of septicaemia was reported by cashew nut consumers in this study.

Enterobacteriaceae counts give indication of the personal hygiene of the workers and the general sanitary practices in and around the factory. The low counts recorded in Tables 2a and 2b could be due to difficulty in isolating these organisms even though they may be in high numbers (Prescott et al; 2006).

The outbreak of *E. coli* 0157:H7 in this study was confirmed apart from stomach upset, nausea, diarrhoea from the lecturers who ate the cashew as snacks served during the second semester examination of 2006, biochemical and serologic tests using slide agglutination test (product DR620, Oxoid, Basingstoke, UK) confirmed the presence of *E. coli* 0157:H7 in ready-to-eat cashew nuts. According to Prescott et al., (2006), *E. coli* are transmitted through faecal oral route and the disease ensures when a contaminated food product or water is ingested.

According to Duffy et al; (2000a), strains of serogroup 0157 produce verocytoxin causing a range of severe to potentially fatal illness. The survival of enterohaemorrhagic *E. coli* sero group 0157 in almost all food types is due to its tolerance to low pH, are able to produce toxins in the intestinal lining regardless of the acidic medium of the stomach (Duffy et al., 2000b).

Fungal counts were the highest amongst organisms counted. They were isolated and characterized macro and microscopically using the methods of Klich (2002) *Aspergillus niger, A. flavus, A. restrictus, penicillium spp. Rhizopus spp.* The high count of these organisms is due to their ubiquitous nature. They explore almost all ecological niche on earth. They are estimated to be responsible for the spoilage of up to 25% of all plant-derived food products annually (Geisen, 1998). There was a significant difference between fungal counts during the raining season and the dry season as could be explained by the m.c shown in Table 1. Most food borne fungi are responsible for high global incidence of mycotoxins which are secondary metabolites and are involved in a toxic response called mycotoxicosis in humans and are the most carcinogenic natural substances known (Geison, 1998; Wild, 2007).

There were undectable levels of aflatoxins during the raining season. Generally aflatoxin production depends on certain factors such as moisture, temperature, presence of toxigenic moulds, suitable substrate for growth, environment conducive for toxin production and other factors such as pH and time (Bertina, 1984; da-Silva et al., 2000).

Although the doses recorded in this study were below the FDA regulatory limit below of 20ppb, recent findings by Oluwafemi and Taiwo(2004), Vismer, (2007) reported that no tolerable daily intake (TDI) can be set for aflatoxin B1. Technically speaking it is impossible to completely eliminate afflatoxing in foods. Therefore new maximum levels have been recently set to follow the principle of As low As reasonably Achievable (ALARA). According Vismer (2007), levels of aflatoxins in foods should not exceed 10ug/kg of which AFB1 < 5ug/kg. The good news is that all cashew samples had less than 5ug/kg of AFB1 (Table 3).

As a result of the outbreak of *E. coli* 0157:H7 infections, improvement of sanitary practices is advocated and addition of a final heating step preceding packaging approved by International Commission of Microbial Specification for Foods (ICMSF)(1996).

In the control of *E. coli* serogroup 0157, an effective control strategy must be adopted that will consider the

multiple points at which *E. coli* 0157 can gain access in cashew nut. Measures to control the bacterium during processing, distribution, at retail level and during commercial/domestic level should be best approached by implementing HACCP principles into the processing safety management system in all stages of the cashew nuts.

Simple sanitary measures should be adopted by workers such as proper disposal of faecal waste, washing of hands with detergent and potable water after defecating and avoiding contact of cashew nuts with raw food products such as meat, vegetable which can serve as vehicle of transfer of *E. coli* 0157. All equipment used in the processing should be kept clean to avoid contact with infected faces or infected animals. Routine sanitary check should be performed on workers to detect if they have pimples, boils that serve as means of introducing *Staphylococcus aureus* into the cashew nuts.

## REFERENCES

**Abe, K., Yamamoto, S. and Shinagawa, K**. (2002). Economic impact of an *Escherichia coli* 0157:H7 outbreak in Japan. Journal of Food Protection 65 (1): 66 – 72.

**Adebayo, L.O and Diyaolu, S.A**. (2003). Mycology and spoilage of retail cashew nuts. African Journal of Biotechnology 2(10): 369 – 373.

**Alice, R.T.** (1994). Cashew nut processing in Southern Tanzania. Eastern African Agricultural Journal 35: 133 – 157.

**Bertina, V**. (Ed). (1984). Mycotoxin: Production, isolation, separation and purification. Elsevier New York, P. 19 – 70.

**da Silva, J.B, Pozzi, C.R, Mallozzi, M.A.B, Ortega, E.M. and Correa, B.** (2000). Mycoflora and occurrence of aflatoxin B1 and fumonisin B1 during storage of Brazilian sorghum. Journal of Agricultural Food Chemistry 48: 4352–4356.

**Duffy, G., Riordan, D.C.R., Sheridan, J.J, Call, J., Whithing, R.C., Blair, S. and McDowell ,D.A**. (2000 a). Effect of pH on survival thermotolerance and verotoxin production of escherichia coli 0157 : H7 during simulated fermentation and storage. Journal of Food Protection 63(1): 12 – 18.

**Duffy, G., Garvey, P. and Sheridan, J.J.** (2000 b). *E. coli* 0157 : H7 : An EU perspective. Irish Journal of Agricultural and Food Research 39: 173 – 183.

**Duff, S.B, Scott, E.A, Mafilios, M.S, Todd, E.C., Krilov, L.R., Geddes, A.M. and Ackerman, S.J.** (2003). Cost-effectiveness in house-hold kitchen to prevent food borne illness in the United States, Canada and the United Kingdom. Journal of Food Protection 66(1): 2103 – 2115.

**Encinas, J.P., Sanz, G.J., Garcia, A.M.R., and Otero, A**. (1996). Evaluation of different systems for the identification of Bacillus strains isolated from Spanish fermented sausages. Meat Science 42(2): 127 – 131.

**Falade, K.O., Akinwande, T.O. and Adedotun, O.O**. (2003). Effect of drying methods on osmotically-dehydrated cashew apples. European Food Research and Technology 216(6): 500 – 504.

**Franscisco, D.C., Oliveira, F. and Lisa, O**. (2002). Bacterial and yeast counts in Brazilian commodities and spices. Brazilian Journal of Microbiology 33: 2 – 10.

**Freire, F., Kozakiewicz, Z. and Paterson, R.M.** (1999). Mycoflora and mycotoxins of Brazilian cashew nuts. Mycopathologia 145: 95 – 105.

**Freire, F. and Bargail, B.M**. (2001). Microbial deterioration of cashew nuts in Brazil. Communication Technology 64: 43 – 48.

**Frenzen, P.D., Drake, A. and Angulo, F.J.** (2005). Economic cost of illness due to *Escherichia coli* 0157 infections in the United States. Journal of Food Protection 68(12): 2623 – 2630.

**Geisen, R.** (1998). PCR methods for the detection of mycotoxin producing fungi in: bridge, P. O., Aroba, D. K., Reddy, C. A., Elander, R. P. Application of PCR in mycology. Oxon, London : CAB International pp 243 – 266.

**Hazariwala, A., Sanders, Q., Hudson, C.R., Hofaacre, C., Thayer, S.G. and Maurer, J.J.** (2002). Distribution of Staphylococcus enterotoxin genes from *S. aureus* isolates from poultry and humans with invasive staphylococcal disease. Avian Diseases 46(1): 132 – 136.

**Henderson, S.** (1985). The relationship between moisture content and equilibrium relative humidity of pig feed. Journal of stored products research 21, 131 – 134.

**Hussein, H. S. and Sakuma, T.** (2005). Shigatoxin – producing Escherichia coli : pre – and postharvest control measures to ensure safety of dairy cattle products. Journal of Food protection 68(1): 199 – 207.

**Johnston, L.M., Jaykus, L.A., Mole, D., Martinez, M.C., Anciso, J., Mora, B. and Moe, C.L**. (2005). A Field study of the microbiological quality of fresh produce. Journal of Food Protection 68(9): 1840 – 1847.

**International Commision on Microbiological Specification for Foods (ICMSF).** (1996). Toxigenic

fungi: Aspergillus. In:Roberts, T.A., Baird-Parker, A.C., Tompkin, R.B. (Eds.), Microorganisms in Foods. Characteristics of Food Pathogens. Blackie Academic & Professional, London, pp. 347-381.

**King, A.D. and Jones, T.** (2001). Compodium of methods for microbiological examination of foods. American Public health Association 57: 561 – 563.

**Klich, M.A**. (2002). Identification of common Aspergillus species 1st ed. Utrecht, the Netherlands : Central Voor Schimmelcultres, pp. 1 – 113.

**Kubo, I., Fujita, K.I., Kubo, A., Nihei, K.I and Lunde, C.S.** (2003). Antibacterial action of ancardic acids against methicillin resistant staphylococcus aures (MRSA). Journal of Agricultural and Food Chemistry 51 (26): 7624 – 7628.

**Laine, E.S., Scheftel, J.M., Boxrud, D.J., Vought, K.J., Danila, R.N., EIfering, K.M. and Smith, K.E**. (2005). Outbreak of Escherichia coli 0157 : H7 infections associated with nonintact blade – tenderized frozen steaks sold by door – to – door vendors. Journal of Food Protection 68(6): 1198 – 1202.

**Lee, W.C., Lee, M.J., Kim, J.S. and Park, S.Y.** (2001). Food-borne illness outbreaks in Korea and Japan studied retrospectively. Journal of Food Protection 64(6): 899 – 902.

**Lee, M.B. and Middleton, D**. (2003). Enteric Illness in Ontario, Canada, from 1997 to 2001. Journal of Food Protection 66(6), 953 – 961.

Leszczynska J, Kucharska U, Zegota H. (2000). Aflatoxins in nuts assayed by immunologic methods. European Food Research and Technology 210 (3): 213 – 215.

**Muniz, C.R**. (2004). Microbial characterization in Brazillian cashew nuts during processing paper presented by the department food technology, Federal University of ceara, Fortaleza, pp 1 – 84.

**Muroi, H. and Kubo, l.** (1993). Bactericidal activity of anacardic acids against streptococcus mutans and their potentiation. Journal of Agricultural and Food. Chemistry 41(10): 1780 – 1783.

**Oluwafemi, F. and Adegoke, G.O**. (2004). Correlation between seasonal variation and microbial deterioration of maize grains stored in different packages. Journal of Food technology 2(4): 293 – 298.

**Oluwafemi, F. and Taiwo, V.O**. (2004). Reversal of toxigenic effects of aflatoxin by on cockerels by alcoholic extracts of African nutmeg, *Monodora myristica*. Journal of the Science of Food and Agriculture 84: 330 – 340.

**Prescott, L.M., Harley, J.P. and Klein, A.D**. (2006). Food-borne Disease, WCB McGraw-hill, pp. 946 – 950.

**Shebuski, J.R., Vilhelmsson, O. and Miller, K.J.** (2000). Effects of growth at low water activity on the thermal tolerance of *Staphylococcus aureus*. Journal of Food Protection 63(9): 1277 – 1281.

**Torquato, D.S**. (2004). Evaluation of antimicrobial activity of cashew tree gum. World Journal of Microbiology and biotechnology 20: 505 – 507.

**Vanselow, B.A., Krause, D.O. and McSweeney, C.S.** (2005). The Shiga toxin – producing *Escherichia coli*, their ruminant hosts, and potential on-farm interventions: a review. Australian Journal of Agricultural Research 56(3): 219 – 244.

**Vismer, H.F.** (2007). Mycotoxin contamination of foods in Africa. Pre and post-harvest paper presented at the second workshop on Food safety held at University of Stellenbosch, South Africa, Nov. 25 – 1st December, 2004 pp. 1 – 54.

**Wild, C.P**. (2007). Aflatoxin exposure in developing countries : the critical interface of Agriculture and health food and nutrition bulletin 28(2): 372 – 380.

**Yoon, Y.H. and Kim, K.I.** (2003). Detection and Identification of beta – lactamase, enterotoxin and other exotoxins genes of *Staphylococcus aureus* by PCR. Asian Australasian Journal of Animal Sciences 16(3): 425 – 429.