

Original Research Article

Microbiological and Parasitological Quality of Local Beef Retailed in Accra and Radiation Sensitivity of *Salmonella* sp

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ABSTRACT

Keywords

Microbiological quality, Beef, *Trichinella* sp, *Salmonella* sp., Irradiation

Microbiological and parasitological quality of raw beef from some retail outlets (local markets, supermarkets, abattoirs) in Accra was assessed and the radiation sensitivity of *Salmonella* sp was determined. Pour plate method, morphological characteristics and biochemical tests were used for enumeration and identification of microorganisms while the muscle digestion method was used to identify the larvae of *Trichinella spiralis*. Radiation sensitivity of *Salmonella* sp isolated from the beef was determined. Total viable counts (TVC) for the local markets, supermarkets and abattoir ranged from 6.36 to 8.47, 5.01 to 8.32 and 5.43 to 8.06(log₁₀ cfu/g) respectively. Coliform counts (CC) for the local markets, supermarkets and abattoirs ranged from 6.14 to 8.35, 2.28 to 8.12 and 1.90 to 5.97(log₁₀ cfu/g) respectively. TVC and CC for the local markets were significantly higher ($P<0.05$) than the supermarkets and abattoirs. TVC and CC for the supermarkets were not significantly different ($P>0.05$) from the abattoir. *Staphylococcus aureus* and *E. coli* counts were not significantly different ($P>0.05$) for the retail outlets. *Salmonella* sp was detected in raw beef from one out of nine retail outlets. None of *Trichinella spiralis* larvae was detected in any sample. Microbiological quality of raw beef from retail outlets are unsatisfactory especially at the local markets due to the high levels of aerobic mesophiles, coliforms, *Staphylococcus aureus*, *E. coli* and isolated cases of *Salmonella* sp contamination. Calculated D_{10} value of 0.43 kGy suggests susceptibility of *Salmonella* sp to medium dose gamma irradiation. Radiation processing of raw beef would enhance hygienic quality of beef in Ghana.

Introduction

Beef is one of the most popular meats in the world and it contributes important nutrients such as high quality proteins, minerals, vitamins and fat to diet. It is particularly rich in iron, zinc, phosphorus and B vitamins. (USDA/ARS/NDL, 2012; Dietary Reference

Intakes, 2006). Despite its benefits, beef has been identified as a potential channel for transmitting food-borne diseases due to its high protein content, high water activity and approximately neutral pH which create favourable conditions for the growth and

survival of bacteria (Bhandare et al, 2007; El-Gohany, 1994; Yousuf et al., 2008)). Conditions of the storage including factors such as temperature and humidity are critical in the growth of spoilage and pathogenic microorganisms on beef, thus the need for effective refrigeration or freezing as primary holding methods (Delmore, 2009, Heinz and Hautzinger, 2007).

Beef products may also be contaminated with the roundworm *Trichinella* larvae by meat grinders and other equipment used for processing pork (Smith and Lechman, 2003) *Trichinella spiralis* has been frequently involved in serious human disease outbreaks (Oivenen et al., 2002).

In Ghana beef constitutes approximately 24% of domestic meat production and 13.6% of imported meat products (MOFA, 2008), indicating a high patronage by consumers. Retailing of beef is predominantly carried out at the local markets, the supermarkets and the abattoirs where different storage and handling practices are adopted for preservation. According to Soyiri et al. (2008), in Ghana slaughtering of animals may be on bare floors outside the abattoir and the carcass may be singed using vehicle tyres and finally washed with unclean water thus facilitating contamination. After slaughtering and initial processing in abattoirs, the beef is chilled immediately and subsequently stored under refrigeration (3-5°C) for retailing purposes.

The meat is transported to the local markets using unapproved and unclean vehicles, while retailing of the beef is carried out in the open under high ambient temperatures exposing the beef to flies and contaminants. Studies have indicated high levels of aerobic mesophilic bacteria and coliforms in khebab from beef and pork (Agbodaze et al, 2005)

and in some cases as much as 15 different bacteria species have been isolated from beef in slaughterhouses in Accra (Mensah et al, 2001). Prevalence of intestinal parasitic infections have been reported among sections of the population in Ghana and these have been related to infected food (Ayeh-Kumi et al, 2009, Esena and Owusu, 2013).

Generally, decontamination of meat products at the industrial level involves the use of non-thermal and thermal technologies such as gamma, electron and X-ray irradiation, high hydrostatic pressure, natural antimicrobials, active packaging, ohmic heating, microwave and radio-frequency as well as steam among others (Aymerich et al, 2008). The use of steam-vacuuming, hot water spray and dilute acids are also recommended procedures for decontamination of meat in slaughter houses (Delmore et al. 1998). Irradiation as a food processing technology possesses unique advantages such as the capacity to decontaminate already packaged foods at ambient temperatures without any loss of sensory or organoleptic properties (Stevenson et al. 1998, Appiah, 1999).

In order to utilize irradiation for decontamination of meat, the determination of radiation sensitivity of contaminating microorganisms is critical.

The D_{10} -value (decimal reduction dose) expresses the radiation sensitivity and this is the dose required to inactivate 90% of a viable bacterial population or reduce the population by a factor of 10. It therefore provides a basis for accurate estimation of inactivation doses (Thayer, 2000; Adu-Gyamfi et al, 2009). Studies have estimated the D_{10} value of pathogens on beef range from 0.11 to 0.8 kGy (Monk et al, 1995; Gramage et al, 1997).

The objectives of this study were to:

- i. Determine the microbiological quality of beef sampled from retail outlets in Greater Accra Region.
- ii. Screen beef sampled from retail outlets in Greater Accra Region for the presence of *Salmonella* sp. and *Trichinella* larvae.
- iii. Determine the radiation sensitivity (D_{10} value) of *Salmonella* sp in beef.

Materials and Methods

Samples

Samples of raw beef were obtained in Accra from local markets (Kaneshie, Dome, Mokola), using simple random selection of retailers while samples from supermarkets (Shoprite, Maxmart, Sotrec) and abattoirs (University of Ghana Farms, Accra Abattoir, and Amasaman Abattoir) were obtained using purposive or judgemental sampling. Each local market, supermarket or abattoirs was sampled on three times.

Microbiological Quality Analysis

Ten grammes of raw beef sample for each retail outlet was homogenized in 90 ml of 0.1% sterile peptone water (1% peptone water and 0.5% Sodium Chloride) with a blender (Waring Laboratory Blender, Christison, Germany) for 5 mins. Microbiological enumeration was carried out using standard decimal dilution and plate count methods (APHA, 1992). Total viable count was estimated on Plate Count Agar (Oxoid, U.K.) for 48 hrs at 35 °C. Coliform count was estimated on Violet Red Bile Agar (Oxoid, U.K.) for 48 hrs at 35 °C. *Staphylococcus aureus* count was estimated on Baird-Parker Agar (Oxoid, U.K.) for 48 hrs at 35 °C. *E. coli* count was estimated on

Eosin Methylene Blue Agar (Oxoid, U.K.) for 48 hrs at 37 °C. *Salmonella* detection was carried out by incubation on Xylose-Lysine-Desoxycholate media (Oxoid, U.K.) for 48 hrs at 37 °C followed by confirmation using the API 20 E microtube system (BioMerieux, France). Each sample was analysed in triplicate and 9 independent experiments were undertaken for each outlet.

Digestion Technique for identification of *Trichinella* larvae in beef striated muscle

One gramme of muscle tissue was cut into pieces and digested in a conical flask containing 20 ml of acid pepsin solution. The mixture was incubated for 24 hrs at 35 – 37 °C and centrifuged (Centurion, U.K.) at 1000 rpm for 4 mins to sediment the larvae. The supernatant was discarded and the sediment was re-suspended in 20 ml formol saline solution and centrifuged again to sediment the larvae. The supernatant fluid was further discarded. The sediment was examined under a light microscope for *Trichinella* larvae. Nine independent experiments were undertaken for each outlet.

Determination of Radiation Sensitivity

Salmonella sp (05 group) isolated from raw beef was used as inoculum. Cultures were reactivated in nutrient broth and incubated at 35-37 °C for 24 hours. The inoculum was standardized to an approximate concentration of 10^7 cfu/ml using serial dilution and pour-plate methods on Xylose-Lysine-Desoxycholate medium (Oxoid, U.K.) for 48 hrs at 37 °C. A 1ml suspension was added to 10 g of freshly slaughtered beef carcass aseptically in low density sterile polyethylene film pouches and sealed. The inoculated beef substrate was homogenized for 3 mins using the stomacher homogenizer (Mix 2 AES Laboratoire, France) and stored

at 3-5 °C for 24 hours. The samples were irradiated at doses of 0, 0.12, 0.24, 0.36, 0.48, 0.60, 0.72, 0.84 kGy at a dose rate of 1.15 kGy/hr in air using a Cobalt-60 source (SLL-02, Hungary).

Fricke dosimetry was used to determine the absorbed dose. The temperature of the samples was maintained between 6 and 9 °C during irradiation using frozen ice packs. All samples were analyzed for surviving *Salmonella* sp. Three replicate experiments were undertaken.

Statistical Analysis

Data obtained from the microbiological survey were subjected to analysis of variance (ANOVA) to determine significant differences between the means. The Statistical Package for the Social Sciences (SPSS version 16, 2007) was used to analyze the data at ($p < 0.05$) significant level. Where there were significant differences, the Tukey's Multiple Comparison test was used to separate the means.

Salmonella counts (cfu/g) were transformed into logarithms and the data subjected to regression analysis to obtain the D_{10} (dose required to inactivate 90% of the population). The D_{10} values were obtained by plotting $\log_{10} N/N_0$ against D (radiation dose) derived from the equation: $D_{10} = D / (\log N - \log N_0)$, where N_0 is the initial viable counts, N the viable counts after irradiation at dose D (Stumbo *et al.*, 1950). The linear correlation coefficient (R^2) and the regression equations were also calculated.

Result and Discussion

Microbiological quality survey

The mean total viable counts (TVC) for raw beef from the local markets, super markets

and abattoirs were 7.91, 6.61 and 6.47 \log_{10} cfu/g respectively (Table 1). Coliform counts (CC) for raw beef from the local markets, super markets and abattoirs gave a mean count of 7.23, 5.20 and 4.18 \log_{10} cfu/g respectively. Both the TVC and CC were significantly ($p < 0.05$) higher at the local markets compared to the supermarkets and abattoirs. There were no significant differences ($p > 0.05$) for the TVC and CC between the supermarkets and the abattoirs.

Mean counts of *E. coli* for beef from the local markets, super markets and abattoirs were within the range of count of 4.80 and 4.53 \log_{10} cfu/g. and those for *Staphylococcus aureus* were within the range of 4.77 and 5.50 \log_{10} cfu/g. No significant differences ($p > 0.05$) were established between the local markets, supermarkets and abattoirs for *E. coli* and *Staphylococcus aureus* counts. No *Salmonella* sp was detected in the each of the nine samples obtained from both the local markets and the abattoirs. Out of the nine samples obtained from the supermarkets, two different groups of *Salmonella* (04 and 05) were detected in one sample.

The mean TVC for the local markets (7.91 \log_{10} cfu/g) exceeded the acceptable standard ($< 7.00 \log_{10}$ cfu/g) specified by both the Ghana Standards Board (GSB) and the International Commission on Microbiological Specifications for Foods (ICMSF). The mean CC for all the three retail outlets also exceeded the acceptable standard of ($< 4.00 \log_{10}$ cfu/g) specified by both the GSB and ICMSF. The study has revealed significantly higher ($p < 0.05$) TVC and CC in raw beef from the local markets compared to the supermarkets and abattoirs, although there were no significant differences ($p > 0.05$) between the TVC and CC in raw beef from the abattoirs and the supermarkets. This suggests probable

differences in the handling, processing and storage of raw beef resulting in relatively satisfactory microbiological quality for beef from the abattoir and supermarkets. The use of refrigeration facilities as well as improved polyethylene packaging and preservatives could probably be the underlying reason for the superior microbiological quality of beef at abattoirs and supermarkets. According to Koutsoumanis and Taoukis (2005) and Delmore (2009), the adoption of proper storage temperature and hygienic as well as effective packaging effectively contribute to improving the safety and quality of raw meat. The results of this study agree with a study by Mathieu *et al.*, (1991) indicating a mean high TVC of 7.00 log₁₀ cfu/g for bovine meat in markets in Zaire due to over-exposure to high ambient temperatures. Ashenafi (1994) also reported a TVC range of 6.00 - 8.00 (log₁₀ cfu/g) in another study on the microbial flora of fresh beef from butcher's shop in Awasa, Ethiopia. The reported TVC values for beef in this study are nonetheless higher compared to the ranges of 4.00 - 5.00 log₁₀ cfu/g by Fliss *et al.* (1991) for Tunisian slaughterhouses and 2.00-4.00 log₁₀ cfu/g by Soyiri *et al.* (2008) for Ashaiman market in Ghana.

Additionally, the mean CC for the three retail outlets (4.18- 7.23 log₁₀ cfu/g) were high compared to 1.00 - 3.00 log₁₀ cfu/g reported by Fliss *et al.* (1991) and Soyiri *et al.* (2008). The mean coliform counts recorded for the supermarket and abattoir however agreed with the range of 3.00- 5.00 log₁₀ cfu/g reported by Ashenafi (1994). The high coliform counts reported for raw beef from the local markets indicated a high level of contamination resulting possibly from the mixing of the offals, the gut and carcass at the local markets (Anderson 1988). According to GSB (1990 b) and Agbodaze *et al.* (2005), most enterobacteriaceae in beef stem from faecal

contamination and their occurrence in high numbers indicate unsanitary handling and/or inappropriate storage conditions for the beef in local markets in Ghana.

The mean counts of *Staphylococcus aureus* and *E. coli* were not significantly different ($p>0.05$) for the three retail outlets. The high levels of *Staphylococcus aureus* (4.77 - 5.50 log₁₀ cfu/g) at the retail outlets indicates the poor handling of raw beef. This finding is important when compared with a related study on poultry meat in supermarkets, local markets and farms in Accra where the mean *S. aureus* were enumerated in the range of 2.28 - 2.70 log₁₀ cfu/g (Adu-Gyamfi *et al.*, 2012). It is probable basic hygienic practices are not observed retailing of raw beef thus resulting in the high *Staphylococcus aureus* counts. The presence of staphylococci in the nasal passages, throats, hair and skin of healthy individuals including food handlers has been reported (FDA/CFSAN, 2007). Although the counts recorded in this study are less than the 5.00 log₁₀ cfu/g required to produce enough enterotoxins to cause staphylococcal food poisoning (FDA/CFSAN, 2007), they raise serious concerns for food safety and related health implications. The high levels of *E. coli* counts (4.35 -4.8 log₁₀ cfu/g) in raw beef from retail outlets could be due to contamination of beef from fecal matter as a result of poor handling of the contents of the intestines during slaughtering (Soyiri *et al.* 2008). These counts compare well with the range of 1.30 – 4.28 x 10⁵ cfu/ml for meat products in markets within the Kumasi metropolis reported by Antwi-Adjei and Maalekuu, 2014. However, counts of *E. coli* reported in the study are far higher than the range of 1.27 -2.74 log₁₀ cfu/g reported by Adu-Gyamfi *et al.* (2012) for poultry meat in super markets, local markets and farms in Accra.

The detection of *Salmonella* 04 and 05 groups in one of the super market beef samples is noteworthy since Ghana Standards Authority recommends absence of *Salmonella* sp in a 25 gramme sample pre-cooked meat (GSB, 1990 a). Other studies have also reported the presence of *Salmonella* sp in raw beef in Ghana. Obeng et al (2013) detected *Salmonella* sp in raw meat sold in the Tolon and Kumbungu districts of the Northern region of Ghana and explained that poor standards of slaughtering and sale of meat are responsible for this observation. In another study by Sackey *et al.*, (2001), detection of *Salmonella* sp in beef was not only attributed to seemingly unhygienic environmental conditions but also to contaminated feed and water. The presence of enteropathogenic bacteria such as *Salmonella* sp and other indicator microorganisms proved the lack of process control under which raw meat products are handled and consequently reveals the potential outbreaks of food poisoning.

Parasitological quality survey

Trichinella larvae were not detected in beef striated muscles obtained from all the retail outlets according to the method used. The absence of *Trichinella* larvae in striated muscles of sampled raw beef obtained from the retail outlets indicate absence of contamination with *Trichinella*. This finding is to be expected since the parasite is mostly found in lightly processed and frozen pork or wild game products (Crompton and Savioli, 2006). The absence of the parasite in raw beef could further be attributed to the feeding system adopted for raising cattle in Ghana and most African countries which relies on extensive open grazing where cattle feed mainly on grasslands, natural pastures and shrubs. Supplementary feeding and the use of concentrates which also tend to be

expensive and notable sources of *Trichinella* are almost non-existent (MOFA, 2008). Cattle are herbivores and consequently not reservoir for *Trichinella*, except when beef products become contaminated through meat grinders and other equipment used for processing pork (Smith and Lechman, 2003). There is therefore the probability for forage to be contaminated with *Trichinella* larvae during handling and processing at the farms (Oivenen, 2002).

Radiation sensitivity of *Salmonella* sp

The results of the radiation sensitivity experiment show that the calculated D_{10} value of *Salmonella* sp (05 group) on raw minced beef at 6 - 9 °C was 0.43 ± 0.02 kGy (Figure 1). The linear correlation coefficient (R^2) was 0.986 and the regression equation was $Y = -2.328X - 0.078$. The coefficient of the regression line was >0.90 , indicating a strong negative linear correlation.

The D_{10} value provides information on the sensitivity of microorganisms to irradiation. The calculated D_{10} value of *Salmonella* sp (05 group) on raw minced beef at a temperature of 6 to 9 °C (0.43 ± 0.02 kGy) compares well with the reported range of 0.371- 0.697 kGy in components of roast beef meal (Grant and Patterson, 1992), 0.38 - 0.77 kGy on chicken at 2 °C (Olson, 1998) and 0.40-0.50 kGy on poultry, pork, eggs and seafood at refrigeration temperature (CAST, 1996). However the mean D_{10} value is lower than the range of 0.55 - 0.78 kGy at 5 °C reported by Smith and Pillai (2004) and 0.519 kGy by Chiasson *et al.* (2004) in ground beef at 5 °C and 4 °C respectively. Sommers and Boyd (2006) reported the average D_{10} for *Salmonella* spp on ready-to-eat beef products as 0.61 kGy. Besides differences in strains of the species and different substrates, the difference in radiation resistance of *Salmonella* sp could

also be due to high water activity in raw beef compared to other beef products (Chirinos, 2002). High D_{10} values for *Salmonella* spp could also be due to the presence of components and other condiments in ready-to-eat foods that compete with bacteria for interaction with free radicals produced during radiolysis of water (Sommers and Boyd, 2006; Chirinos, 2002). According to Thayer (2004), physical and chemical composition of food also affects microbial responses to irradiation.

The calculated D_{10} value of 0.43 kGy for *Salmonella* sp on raw beef practically means the minimum of current approved doses of 5-7kGy for elimination of pathogens on raw beef (Ghana Standards Authority, 2007) will achieve a minimum of 11 log cycle reduction. Consequently, radiation processing of raw beef in Ghana for the

purpose of eliminating pathogenic strains of *Salmonella* sp can be seen as a highly effective processing technology that would safeguard the hygienic quality of beef and possibly other meat products.

Microbiological quality of raw beef from retail outlets is unsatisfactory especially at the local markets due to the high levels of aerobic mesophiles, coliforms and potential pathogens like *Staphylococcus aureus*, *E. coli* and isolated cases of *Salmonella* sp contamination. The calculated D_{10} value of 0.43 kGy suggests susceptibility of *Salmonella* sp to medium dose gamma irradiation. Application of approved doses for radiation processing of raw beef for the purpose of eliminating pathogens such as *Salmonella* sp would enhance hygienic quality of beef and other meat products.

Table.1 Microbial counts of raw beef from different retail outlets

	Retail outlets		
	Local Market	Supermarket	Abattoir
Total Viable Count	7.91 ^a ±0.75	6.61 ^b ±1.18	6.47 ^b ±0.76
Coliform Count	7.23 ^a ±0.57	5.20 ^b ±1.76	4.18 ^b ±1.14
<i>E. coli</i>	4.80 ^a ±0.78	4.53 ^a ±1.30	4.35 ^a ±1.51
<i>S. aureus</i>	5.50 ^a ±0.97	4.77 ^a ±1.25	4.87 ^a ±1.27

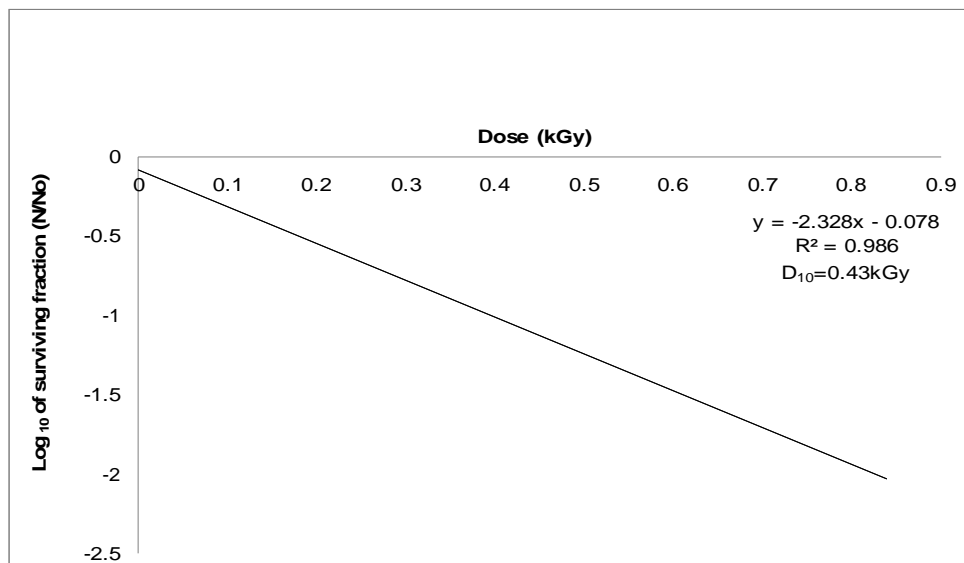
Values are mean counts of nine independent experiments. Counts are expressed as log 10 cfu/g. Means in a column bearing the same superscript are not significantly different $p > 0.05$. ND- Not detected. D- Detected.

Table.2 Presence of *Salmonella* sp. and *Trichinella* larvae in raw beef from different retail outlets

Pathogen/Parasite	Retail outlets		
	Local Market	Super Market	Abattoir
<i>Salmonella</i>	ND	D (one out of nine)	ND
<i>Trichinella larvae</i>	ND	ND	ND

Results of 9 independent experiments; ND- Not detected; D- Detected.

Figure.1 Radiation resistance curve of *Salmonella* sp on raw minced beef



Acknowledgement

The author is grateful to Messrs. Caleb Owulah, E. Akolmoga, and Daniel Larbi of BNARI, Ghana Atomic Energy Commission and Mr. Jonas Asigbee of Noguchi Memorial Institute of Medical Research, Department of Parasitology for their technical support.

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