The microbiological quality of imported frozen chicken drumsticks from retail meat shops in Accra, Ghana

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ABSTRACT

There has been an increase in chicken meat consumption in Ghana due to the affordability and convenience of imported frozen chicken parts compared with fresh chicken meat. However, there is little information on the quality of frozen chicken meat. The aim of this study was to assess the microbiological quality and safety of imported frozen chicken drumsticks from supermarkets, cold stores and open markets. A total of 24 chicken meat samples were analyzed to determine the Total Viable Count (TVC), Total Coliform Count (TCC), concentration of *Escherichia coli* and prevalence of *Salmonella*. The mean TVCs were 6.34, 5.92 and 5.42 Log CFU/g respectively for open markets, supermarkets and cold stores. *E. coli* and *Salmonella* prevalence was 66.7% and 91.7% respectively. There was no significant difference in the microbiological quality and prevalence of *E. coli* and *Salmonella* on frozen chicken meat purchased from all three retail outlets. Results of this study suggest that raw frozen chicken drumsticks from Ghanaian retail outlets have poor microbiological quality and are highly contaminated with potential pathogens including species of *E. coli* and *Salmonella*.

Keywords: Chicken, Microbiological quality, food safety, *Salmonella, Escherichia coli*

Introduction

Chicken meat is an important component of the Ghanaian diet. Its consumption has risen steadily since the year 2000 (GAIN, 2011) and now comes second to fish in the supply of animal proteins in the Ghanaian diet. In 2011, poultry consumption was 115,000MT, up from 108,000MT the previous year. Yet, domestic supply of broilers meets less than 10% of the local demand. According to the GAIN (2011) report, 90% of chicken meat consumed locally is imported mainly in the frozen state. In the urban and peri-urban areas, imported frozen chicken parts are highly patronized by operators of hotels, restaurants, fast food joints and for home consumption. The high patronage is due to the lower cost of such imported chicken parts compared to locally produced broilers. Besides, they are sold 'pre-cut', making them convenient to use. The drumsticks are particularly popular.

Freezing is usually the method of choice for long term preservation of meat, and is known to cause 1 to 2 Log CFU/g reductions in microbiota, especially during slow freezing (Adam and Moss, 2008; Adu-Gyamfi *et al.,* 2014). However, freezing can give a false sense of quality and safety if the cold chain is not properly maintained. The microbiology of chicken meat at the point of sale depends on several factors including method of slaughter, sanitation during processing, packaging and dispatch, hygienic handling at retail, and maintenance of the cold chain from slaughter to retail (Selvan *et al.,* 2007). Contamination of raw chicken meat along the value chain can be aggravated during temperature abuse.

In Ghana, frozen chicken parts are marketed in supermarkets, cold stores and open markets that have the infrastructure to support cold storage. Typically, frozen meat is purchased in bulk and transported at ambient

temperatures to retail shops. As frozen meat begins to thaw, condensation on the meat surface supports the multiplication of both spoilage and pathogenic microorganisms, which affect the microbiological quality of the chicken meat. Other microbiological risk factors at retail points include frequent opening and closing of freezers, which increases the freezer temperature and thus compromises the cold chain. Direct contact of frozen meat with ungloved hands, and often after handling retail items such as money, could lead to non-source contamination. Furthermore, with a poorly resourced power sector, even small and medium scale retailers who own generators do not power their freezers throughout the period of power outages. All these have implications for the quality and safety of frozen chicken meat at the point of sale.

Presently, limited information exists on the quality of frozen chicken in the Ghanaian retail market. Arhin Sackey *et al*. (2002) assessed the presence of *Campylobacter*, *Salmonella*, *Shigella* and *Escherichia coli* in live and dressed poultry from Accra, reporting <15% prevalence of the targeted pathogens. Adu-Gyamfi *et al.,* (2014) also reported the presence of *E. coli* on raw chicken meat. Therefore, frozen chicken meat on the Ghanaian market needs to be frequently monitored to safeguard the safety of the populace and design policies and interventions to control such meat and prevent enteric foodborne diseases.

The aim of this study was to assess the microbiological quality and the prevalence of *E. coli* and *Salmonella* on imported frozen chicken drumsticks from Ghanaian retail shops.

Materials And Methods

Sample collection

Twenty-four packs of frozen chicken drumsticks were purchased from twelve retail shops in Accra and Tema metropolis. The retail shops comprised four supermarkets, four cold stores and four open markets with functional freezers. For each of the shops, a pack of chicken drumsticks was purchased on two different days. Each sample was placed in a sterile plastic bag and transported immediately to the laboratory in a disinfected thermos flask. Frozen chicken was allowed to thaw at refrigeration temperatures prior to microbiological testing.

Microbiological analyses

A 25g portion of chicken was excised aseptically and placed in a sterile stomacher bag containing 225ml of sterile buffered peptone water (Oxoid, England). The sample was homogenized for 2 minutes in a stomacher and the homogenate used for further serial dilutions with sterile buffered peptone water as diluent. Microbiological tests were conducted using standard microbiological methods (ISO 4833-1 for total viable count, ISO 4832:2006 for total coliform count, ISO 16654:2001 for *Escherichia coli*, and ISO:6579:2002 for *Salmonella* spp.). Total aerobic mesophiles were determined on Plate Count Agar (Biolab, USA) incubated at 37˚C for 24 h. To enumerate Total coliforms, Violet Red Bile Glucose Agar (Oxoid, England) was used and cultured at 37˚C for 24 h. *Escherichia coli* was detected using Eosin Methylene Blue Agar (Oxoid, England) incubated for 24h at 45˚C. Colonies with metallic sheen were enumerated as presumptive *E. coli*. Presumptive *Salmonella* was detected using Brilliant Green Agar (Oxoid, England) at 37˚C for 24 h. Red colonies with imparted red/pink colour in the surrounding medium were considered typical colonies of *Salmonella*. For all microbiological testing, the pour plate method was used to determine microbial contamination on frozen chicken samples. Characteristic colonies enumerated were reported as CFU/g. Presumptive colonies of *E. coli* and *Salmonella* were confirmed with the following biochemical tests: Triple Sugar Iron (TSI) test, Indole test, catalase test, citrate test, and Gram test for *E. coli*; and Triple Sugar Iron (TSI) test, Catalase test, Citrate test, urease test, lysine decarboxylase test and Gram test for *Salmonella* spp.

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to determine whether there were statistical differences between the microbiological quality of frozen chicken drumsticks from the different categories of retail shops. P value was set at ≤0.05. Samples were analyzed in duplicate. ANOVA was carried out to detect significant differences between counts from the three types of retail outlets using Minitab 14.

Results and Discussion

The mean bacterial counts and prevalence of *Salmonella* in raw frozen chicken drumsticks are presented in Table 1. The total viable bacterial counts ranged from 5.40-7.48 Log CFU/g, 3.78-6.67 Log CFU/g and 3.00-6.92 Log CFU/g for open markets, supermarkets and cold stores respectively. There were no significant differences (P> 0.05) between mesophilic counts between all three types of retail outlets. Summaries of confirmatory tests for *Salmonella* and *E. coli* are presented in Tables 2 and 3 respectively. With the exception of one open market sample, most of the counts were within the national specification of <7 Log CFU/g. The level of compliance of the frozen chicken samples to national standards is presented in Table 4. The upper limits for mesophilic bacteria on frozen chicken drumsticks were generally higher than those reported by Alvarez-Astorga *et al.* (2002) who recorded counts in the range of 4.97- 5.79 Log CFU/g for raw chicken legs. Higher mesophilic bacterial counts have been reported in other studies. For instance, vacuum packaged refrigerated ostrich fillets were found to contain 6.20-7.78 Log CFU/g bacteria, while a mean aerobic count of 7.70 Log CFU/g was reported for frozen chicken meat (Alonso-Calleja *et al.,* 2004: Javanmard *et al.,* 2006). High bacterial loads were attributed to possible temperature abuse during processing, storage, transportation, distribution or retail. Similarly, high mesophilic counts recorded for frozen chicken drumsticks in this study could be due to a lack of integrity of the cold chain from dispatch, transportation, and especially storage, and cross-contamination from handling at retail. Transportation at ambient temperatures is a common practice in Ghana, which, in such a tropical environment, could reach well beyond 30˚C. Several freeze-thaw cycles could have resulted in microbial proliferation. The poor microbial quality of meat observed could also be due to cross-contamination from other unpackaged meats usually stored together in the freezers. Cross-contamination with hands of personnel handling different types of meat and other non-meat retail items, including money (currency notes), are all possible sources of microbial contamination. Moreover, repeated opening and closing of freezers can result in fluctuations of freezing temperatures and may allow phychrophiles such as *Listeria monocytogenes* which has a growth temperature range of -1.5 to 45°C to proliferate, albeit slowly (FSANZ, 2017; Ryser and Donnelly, 2015).

The presence of mesophilic bacteria is an index of the hygienic quality of meat and also represents a significant portion of spoilage bacterial populations on the meat. Biochemical changes such as sliminess and putrefaction which are evidence of microbial spoilage of meat are often masked when meat products are frozen. Such changes become evident when microbial populations reach 7 log CFU/g and above. More than 66% of the frozen chicken samples in this study had total viable bacterial counts >6.0 Log CFU/g (Table 1). Temperature fluctuations due to frequent opening and closing of freezers and multiple freeze thaw cycles because of power fluctuations could lead to quality deterioration of chicken meat as a result of microbial growth of psychrophiles during temperature abuse.

Table 1: Bacterial counts and prevalence of *Salmonella* on frozen chicken drumsticks

Market type	Market samples	Log_{10} CFU/g (±Standard deviation)			
		TVC	TCC	E. coli	*Salmonella* detection
Open Air Market	OP1	6.18 (±0.94)	5.10(±2.55)	2.88(±1.56)	++
	OP2	6.44 (±1.47)	4.00(±0.99)	4.00(±0.99)	++
	OP3	6.18(±0.52)	5.89(±0.41)	3.12(±2.23)	++
	OP4	6.55(±0.08)	2.90(±0.57)	2.54(±0.51)	+
Supermarket	SP1	5.22(±2.04)	3.69(±3.38)	0.59(±0.83)	++
	SP2	6.04(±0.37)	2.32(±0.40)	0.85(±1.20)	++
	SP3	6.37(±0.27)	4.47(±2.03)	4.54(±2.31)	+
	SP4	6.05(±0.44)	4.85(±0.21)	0.77(±1.09)	++
Cold store	CS1	6.65(±0.37)	3.51(±2.78)	0.50(±0.71)	++
	CS2	4.24(±1.75)	2.36(±0.31)	2.70(±3.82)	++
	CS3	5.70(±0.06)	3.57(±0.24)	1.74(±2.46)	++
	CS4	5.09(±0.12)	3.20(±0.28)	1.06(±1.49)	++
	P value	0.181	0.271	0.499	

TVC: Total Viable count; TCC: Total Coliform Count; +detection on one sample, ++ detection on 2 samples from the same market

Table 2: Summary of confirmatory test results for *Salmonella* isolated from frozen chicken samples

Sample ID	Triple Sugar Iron				Catalase	Citrate	Urease	Lysine De-carboxylase	Gram's test	Morphology
	Butt	Slant	H_2S	Gas						
OP1	+	+	+	+	+	+	-	+	-	Rods
OP2	+	+	+	+	+	+	-	+	-	Rods
OP3	+	+	+	+	+	+	-	+	-	Rods
OP4	+	+	+	+	+	+	-	+	-	Rods
CS1	+	+	+	+	+	+	-	+	-	Rods
CS2	+	+	+	-	+	+	-	+	-	Rods
CS3	+	+	+	+	+	+	-	+	-	Rods
CS4	+	+	+	+	+	+	-	+	-	Rods
SM1	+	+	+	+	+	+	-	+	-	Rods
SM2	+	+	+	-	+	+	-	+	-	Rods
SM3	+	+	+	+	+	+	-	+	-	Rods
SM4	+	-	-	+	+	+	-	+	-	Rods

OP1-4: Open markets at four locations in Accra; CS1-4, Samples from Cold Stores at four locations in Accra; SM1-4: Samples from supermarkets at four locations in Accra. From each sample location, six presumptive colonies were tested. Presented here are summary results for at least one positive colony for Salmonella from frozen chicken sampled from each of the sample locations.

Table 3: Summary of confirmatory tests for *Escherichia coli* isolated from frozen chicken obtained from markets in Accra

Sample ID	Triple Sugar Iron				Catalase	Indole	Citrate	Gram's test	Morphology
	Butt	Slant	H_2S	Gas					
OP1	-	+	-	+	+	+	-	-	Rods
OP2	-	+	-	+	+	+	-	-	Rods
OP3	-	+	-	+	+	+	-	-	Rods
OP4	-	+	-	+	+	+	-	-	Rods
CS1	-	+	-	+	+	+	-	-	Rods
CS2	-	+	-	-	+	+	-	-	Rods
CS3	-	+	-	+	+	+	-	-	Rods
CS4	-	+	-	+	+	+	-	-	Rods
SM1	-	+	-	+	+	+	-	-	Rods
SM2	-	+	-	+	+	+	-	-	Rods
SM3	-	+	-	+	+	+	-	-	Rods
SM4	-	+	-	+	+	+	-	-	Rods

OP1-4: Open markets at four locations in Accra; CS1-4, Samples from Cold Stores at four locations in Accra; SM1-4: Samples from supermarkets at four locations in Accra. From each sample location, six presumptive colonies were tested. Presented here are summary results for at least one positive colony for *E. coli* from frozen chicken sampled from each of the sample locations.

Javanmard *et al.* (2006) reported a mean coliform count of 7.00 Log CFU/g for frozen chicken meat, which was higher than the values obtained in this study. On the other hand, coliform counts from this study are supported by similar studies by Adu-Gyamfi *et al.* (2012) who reported mean coliform counts of 3.80 Log CFU/g for supermarkets, 3.46 log CFU/g for local markets and 3.14 log CFU/g for chicken carcass obtained from farms. Coliforms are good indicators for the general sanitary condition of the food and may suggest hygiene gaps in food processing environments and in processed foods.

Table 4: Compliance of frozen chicken drumsticks to national microbiological standards

Market type	N	Mean counts (Log_{10} CFU/g) (% Compliance to Standard)			
		TVC	TCC	E. coli	Salmonella
Open market	8	6.34 (87.5%)	4.47 (50.0%)	2.56 (12.5%)	7* (12.5%)
Supermarket	8	5.92 (100%)	3.90 (50.0%)	1.69 (37.5%)	7* (12.5%)
Cold Store	8	5.42 (100%)	3.16 (87.5%)	1.50 (50.0%)	8* (0%)
National Standard		< 7.0[a]	<4.0[b]	ND[c]	ND[d]

TVC: Total Viable count; TCC: Total Coliform Count, *Number of samples positive for *Salmonella*, Ghana Standard reference numbers a: GSS955/2013; b: GS 236:1997; c: GS 955/2009; d: GS 955/2013.

The presence of *E. coli* (fecal coliforms) on the other hand suggests recent fecal contamination. In this study *E. coli* had a prevalence rate of 67% while *Salmonella* prevalence reached 92% (Table 2). There were no significant differences in prevalence of *E. coli* and *Salmonella* between open markets, cold stores and supermarkets (P> 0.05). Similar prevalence has been reported for *E. coli* on chicken (Arhin Sackey *et al*., 2001; Adu-Gyamfi *et al*., 2012; Odwar *et al*., 2014; Sharma and Chattopadhyay, 2015). The presence of *E. coli* on frozen chicken may suggest poor personnel hygiene and unsanitary contact with carcasses along the value chain.

Salmonella prevalence generally exceeded figures reported in the literature. Adu-Gyamfi *et al.* (2012) reported a *Salmonella* prevalence of 7% on raw chicken meat in Accra, Ghana, whereas Arhin Sackey *et al*. (2001) reported a 13% prevalence in freshly dressed chicken carcasses in the same city. These discrepancies could be due to the fact that fresh or chilled poultry carcasses, which the above authors investigated, have shorter shelf-lives, and thus experience less handling and storage during processing and retail compared to frozen meat. Although the association of *Salmonella* with chicken has been well established (Vindigni *et al*., 2007; Adam and Moss, 2008), the high prevalence obtained in this study raises concerns about food safety.

Conclusions

The results of this study highlighted the issue of poor microbiological quality of frozen chicken drumsticks at the point of sale. The high prevalence of potential foodborne pathogens is of great concern. It is recommended that regulatory authorities work closely with stakeholders in the frozen chicken trade to set performance objectives at different stages in the value chain and employ control measures that will effectively preserve chicken meat until use.

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