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Bacteriological Quality of Beef Offered for Retail Sale in Cote d'ivoire

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ABSTRACT

This study was designed to monitor the bacteriological quality of beef meat produced and offered for retail sale on the different markets of Abidjan after slaughtering beefs in the main slaughter-house of Abidjan (Côte d'Ivoire). The bacterial load was assessed on samples collected at a regular 3 h interval from 6: am to 6: pm according to classical methods for the examination of foods. Mean counts (log₁₀ cfu g⁻¹) of total aerobic microorganisms, faecal coliforms, Staphylococcus aureus, Pseudomonas, were 4.93, 1.83, 1.53 and 1.29 at 6: am and at 8.1, 4.73, 2.43 and 2.79 at 6: pm, respectively. The pH ranged from 5.09 to 6.90 and the temperature from 37.29 to 39.59°C. Anaerobic sulphite reducers were detected only at 3: pm and 6: pm with count ranging from 0.76 to 0.83 log₁₀ cfu g⁻¹. Salmonella was detected in 7 to 27% of the samples analyzed. All the counts exceeded the established values in the microbiology criteria for beef meat. Based on the quality criteria established for beef meat, the percentage of samples of a satisfactory quality decreased within the same day to reach 0% in the evening. The percentage of an acceptable quality decreased from 47% at 6: am to 0% at 12:00 noon while that of an unacceptable quality increased from 33 to 100% at 6: pm. All the samples were contaminated at the end the selling time (6: pm). Therefore, the product was unsafe for human consumption. The presence of these organisms is a warning signal for a possible occurrence of food intoxication.

Key words: Beef meat, retail sale, slaughter-house, bacteriological quality, time

INTRODUCTION

Developing countries face with high incidences of food poisoning outbreaks, with obvious economic consequences. While food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with foods from animals (Kivi et al., 2007; Maripandi and Al-Salamah, 2010). Meat is considered as the most important source of proteins consumed by humans. However, meat is the most perishable of all staple foods since it contains sufficient nutrient needed to support the growth of microorganisms (Huda et al., 2010). For highly perishable foodstuffs such as fresh red meat, the threat of food poisoning is particularly high (Nel et al., 2004; Yousuf et al., 2008). Thus, if food is not immediately utilized or preserved after harvesting, it spoils. Côte d'Ivoire is a wet sub-Saharan African country where bovine cattle breeding are almost absent. So the country imports livestock up to 80% of its needs

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from Mali and Burkina Faso. Although beef meat is popular in Côte d'Ivoire, it accounts for 37% of the meat consumed (Anonymous, 2005) because of the increasing price of meat products.

In Côte d'Ivoire, fresh meats are sold everyday at a retail level to the public on open markets especially for low-income people who can't afford a refrigerator for preservation. After slaughtering, beef carcasses are not stored in chillrooms. So they must be sold within the same day. It is generally agreed that the internal tissues of healthy slaughtered animals are free of bacteria at the time of slaughtering. However, when the meat gets to the retail market, it may contain varying numbers and types of microorganisms (Nollet and Boylston, 2007). In addition, in Côte d'Ivoire, food such as fish or fresh meat is always kept exposed while awaiting buyers, making it naturally vulnerable to infection with different types of microorganisms. Therefore, improper handling and improper hygiene may lead to the contamination of fresh meats and eventually affect the health of the consumers (Koussemon et al., 2008).

In Côte d'Ivoire, even though people were used to well cook food and ate home most of the time, now-a-days, the alimentary trend has changed. Many people eat out buying ready to eat foods sold by the street sides. It should be noted that these foods are undercooked most of the time. In the world, one of the most important food safety hazards is associated with undercooked meat and poultry (Dyckman and Lansburgh, 2002). Street vending of foods is a common characteristic of countries with high unemployment rates, low salaries and poor social security programme. In West Africa, especially in Côte d'Ivoire, many popular street-fast-processed and vended foods such as chicken and beef meat are not always well cooked and are eaten without further processing or cooking. Therefore, contaminated foods, from fresh red meat infected with microorganisms, can lead to consumer health problems. Ologhobo et al. (2010) observed that microbial counts of chicken and beef suya (street sides roasted meat) were at levels that pose health problems to consumers. The same authors reported earlier on pathogens like Salmonella, Staphylococcus and entheropathogenic Escherichia coli in beef suya.

There are relatively few surveys and a lack of information on the bacteriological status of beef carcasses offered for retail sale and the level of hygiene of the slaughterhouses in Côte d'Ivoire. Since the beef carcasses are not preserved after slaughtering but sold within the same day at ambient temperature, it seems important to investigate some bacteria such as *E. coli* and *S. aureus* which are indicators of excessive human handling (Clarence et al., 2009). Pseudomonas, a proteolytic contaminating food bacterium which may be used to predict the level of spoilage of the beef carcasses while several foodborne outbreaks linked to Salmonella were attributed to meat and poultry (Dechet et al., 2006; Thomas et al., 2006). The presence of Salmonella infections such as typhoid fever continues to be a major public health concern in many countries such as Côte d'Ivoire. Therefore, the aim of this study was to assess the evolution of such bacteria over the time and within the same day of slaughtering. The bacteria investigated were total aerobic count, anaerobic sulphite reducers, Staphylococcus aureus, Pseudomonads, faecal coliforms and Salmonella.

MATERIALS AND METHODS

Material and sampling: Beef carcasses were obtained from the main slaughterhouse in Abidjan, located in the commune of Port-Bouet, Abidjan District, Côte d'Ivoire from january to june 2009. Beef carcasses were sampled at the end of the slaughtering because they were not stored in chillrooms but exposed at ambient temperature (32±4°C). The samples were collected with 3 butchers chosen randomly in the slaughterhouse. Per sampling day, samples of about 300 g were aseptically collected in a clean polyethylene bag from the same portion (the thigh) of the same

carcass from each butcher using the excision method as indicated by Gill and Jones (2000). The thigh was sampled from all the vendors on Monday mornings at 3 h intervals between 6: am (end of slaughtering) and 6: pm (end of selling). The average time interval between sampling from one butcher to the next one was ten minutes. A total of 300 samples were collected from 60 beef carcasses. Each sample was placed into a sterile plastic bag, labelled and stored in an ice box waiting to be shipped to the laboratory.

Temperature and pH determinations: The temperature and pH of the meat samples were monitored *in situ* to the abattoir using a portable thermometer. The measures were determined less than 1 h post-mortem and monitored at 3 h intervals for 12 h from 6: am to 6: pm. The temperature and pH were obtained at the time of sampling by inserting a thermometer and a probe electrode into the muscle.

Microbiological assays: Once in the laboratory, no more than 2 h after sampling, each sample was cut into smaller pieces and ground in a stomacher into a homogeneous mixture. Twenty five grams of the mixture were suspended in 225 mL of 0.1% sterile buffer peptone water (Oxoid CM 509) in a plastic bag and mixed together again for 30 sec at normal speed. The homogenates were used for all the microbiological analyses. The 1 mL of the homogenate was serially diluted in an aseptic condition and used for the enumeration of microorganisms. Total aerobic counts were performed using Plate Count Agar (Oxoid CM 463); faecal coliforms and Staphylococcus spp counts were carried out on violet Red bile lactose agar (Oxoid CM 107), incubated at 44°C for 24 h and Baird Parker Agar (Oxoid CM 275) at 37°C for 24-48 h, respectively. Typical black colonies with zones around and atypical black colonies were considered as Staphylococci spp. Staphylococcus aureus was then identified using coagulase test, DNase test, Gram reaction and catalase activity. For the isolation of an aerobs that are capable of reducing sulphide, Trypsin Soja Neomycin (TSN, Merck 1.11972) was inoculated and incubated at 37°C for 24-48 h. We investigated Pseudomonas spp. on Cetrimide Agar (Biorad, France) at 30°C for 24-48 h. Oxidase (+) colonies were taken into consideration. Salmonella spp. were isolated using the classical technique with a pre-enrichment on buffered peptone water (Oxoid CM 509), enrichment into Rappaport Vassiliadis Broth (Merck 1. 07700), isolation on Salmonella-Shigella Agar (Merck 1.07667) and identification with the API 20 E (Biomérieux, France). The presence or absence of Salmonella was taken into consideration. The mean number of colonies counted for all count types (except Salmonella) was expressed as \log_{10} colony forming units per gram (cfu g⁻¹) of sample. All the tests were carried out in duplicate. The expressions "acceptable, marginally acceptable, unacceptable" were used to determine quality of the samples according to the microbiological criteria for fresh beef meat (Clarence et al., 2009).

Statistical analysis: The data collected underwent an Analysis of Variance (ANOVA) using STATISCA 99. All values were expressed as the mean of three measurements. The data collected were subjected to one way Analysis of Variance (ANOVA). With respect to the bacteriological level, temperature and pH investigation for each to sampling time, mean values were compared for the different sampling times. Duncan test was used in order to determine which means were significantly different from which others (p = 0.05).

RESULTS

Temperatures and pH of samples: The mean temperature and pH values at various times post mortem are presented in Fig. 1. The temperature ranges from 37.29 to 39.59°C. The pH values

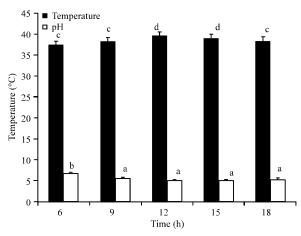


Fig. 1: pH and temperatures of beef carcasses at different sampling times. Bars with same letter are not significantly different

Table 1: Bacterial mean counts of beef carcasses analysed at different sampling times

Mean counts (log₁₀ cfu g⁻¹)

Time	TAC	FC	ASR	S. aureus	Pseudomonas	Salmonella
6 : am	4.93 ± 1.78^{b}	1.83 ± 0.15^{a}	0	1.53±0.30 ^a	1.29 ± 0.36^{a}	+
9 : am	$5.40\pm0.62^{\circ}$	3.20 ± 0.30^{ab}	0	2.76 ± 0.45^{ab}	1.36 ± 0.20^{a}	+
12:00	7.36 ± 1.33^{d}	3.53 ± 0.58^{ab}	0	$3.46{\pm}0.47^{ab}$	1.63 ± 0.49^{a}	+
3 : pm	7.50 ± 1.83^{d}	4.80 ± 1.39^{b}	0.76 ± 0.11^{a}	3.59 ± 0.52^{ab}	2.09 ± 0.52^{a}	++
6 : pm	8.10±1.60°	4.73 ± 1.35^{b}	0.83 ± 0.25^{a}	2.43±0.51ª	2.79 ± 0.30^{a}	+++

TAC: Total aerobic count, FC: Faecal coliforms, ASR: Anaerobic sulphide-reducers, +: Present in less than 10% of samples, ++: Present in 20% of samples, +++: Present in 27% of samples. Means with the same letter in the same column are not significantly different at 5%

range from 5.09 to 6.90 with a mean value of 5.7. There was no significant difference (p>0.05) between mean temperatures at 6: am and 9: am and between 12: 00 and 3: pm. However, there was a significant difference (p<0.05) between mean temperatures at 3: pm and 6: pm. concerning the pH, no significant differences (p>0.05) was observed from 9: am to 6: pm. But there was one (p<0.05) between 6: am and 9: am. It should be emphasized that the lowest pH value was recorded at the highest temperature (39.59°C).

Bacterial loads of samples: The distribution pattern of the bacteria counts are shown in Table 1. The mean of total aerobic count on the fresh meat ranged between $4.93\text{-}8.1 \log_{10} \text{ cfu g}^{-1}$, faecal coliforms count between $1.83\text{-}4.73 \log_{10} \text{ cfu g}^{-1}$, S. aureus between $1.53\text{-}2.43 \log_{10} \text{ cfu g}^{-1}$ while Pseudomonad's count ranged between $1.29\text{-}2.79 \log_{10} \text{ cfu g}^{-1}$. The bacterial counts increased with time for all the samples analyzed. Mean counts $(\log_{10} \text{ cfu g}^{-1})$ of total aerobic bacteria, faecal coliforms, S. aureus, Pseudomonas were at 6: am 4.93, 1.83, 1.53, 1.29 and at 6: pm 8.1, 4.73, 2.43, 2.79, respectively. The anaerobic sulphide reducers were not detected during the first periods of sampling from 6: am to 12:00. Their counts started at 3: pm with values ranging from 0.76 to $0.83 \log_{10} \text{ cfu g}^{-1}$ at the end of the selling time. Significant differences (p<0.05) were observed between samples for total aerobic counts, faecal coliforms and S. aureus from 6:am to 6:pm. However, no significant difference (p>0.05) was observed between samples for Pseudomonas and the anaerobic sulphide reducers for all the sampling times. Differences in counts up to $2 \log$ units were found at

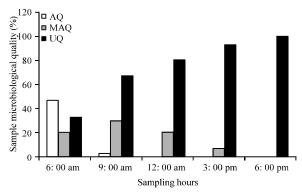


Fig. 2: Percentage of the microbiological quality of the samples at different sampling times (AQ: Acceptable quality, MAQ: Marginally Acceptable quality, UQ: Unacceptable quality)

the same time period when compared to different carcasses. Salmonella was detected in 7 to 27% of the samples analyzed.

The bacteriological quality of all the beef samples analysed is represented in Fig. 2. The percentage of acceptable quality decreased from 47% at 6: am to 0% at 12:00 while that of the unacceptable quality increased from 33 to 100% at 6: pm. At 9: am, only 3% of the samples were acceptable for human consumption. All the samples were contaminated at the end the selling time (6: pm) and no sample was even marginally acceptable for human consumption.

DISCUSSION

The different temperature values recorded in this study were in accordance with those reported by Mackey and Roberts (1993) who indicated that once the slaughtering is complete, the internal temperature of the carcasses generally ranges between 30 and 39°C. The temperature of the muscle tissue in this study falls slowly probably because the beef carcasses were exposed to ambient temperatures above 30°C and possibly to some radiant heating. In this study, the lowest pH was recorded at the highest temperature. This result agreed with that of Bowker et al. (2010) who stipulated that a rapid decrease of pH values at higher temperature may burst the lysosomal membrane in which some cathepsins could hydrolyze specific myofibrillar proteins. Also, the combination of low pH and high temperature could provoke an earlier release of Ca²+ from the sarcoplasmic reticulum, thus activating calcium-dependent protease-I which retains 28% of its maximum activity at post-mortem conditions of pH 5.5 to 5.8. The mean pH value of 5.7 found in this work falls in these pH conditions. The lowest pH value at the highest temperature suggested an increasing production of lactic acid via an aerobic glycolysis during marketing at elevated temperature. Huff-Lonergan and Lonergan (2005) reported that high postmortem temperatures enhanced the degradation of muscle proteins. The highest mean pH values obtained at 6: am in this work probably indicate differences in pH of the groups of muscles in the portions cut from carcasses at different times. Ndou et al. (2011) reported that physical activity of the animals for hours before slaughter, reduces glycogen concentration and plasma glucose levels below critical values and eventually leading to increased meat pH above critical range of 5.5 to 6.0. This argument could explain the pH values in this work which range from 5.09 to 6.90.

The mean temperatures of beef carcasses obtained over the time suggest that the meat was exposed to bacteria proliferation. The high level of microbial flora on the meat from this study could be generally attributed to the filthy environment, poor personal hygiene of the butchers and retailers. There could be possible cross contamination between adjacent raw meat through unclean

hands of the handlers and/or flies. The increasing numbers of bacteria recovered from samples over time could be due to growth and/or increasing numbers of bacteria being deposited on the carcass along with dust. Also, the hands and knives, gloves, aprons, cutting table surfaces used could be implicated as media for cross contamination of the meat and spread spoiling organisms such as *Pseudomonas* and pathogens present on carcasses onto freshly exposed tissues and meat from other carcasses. Likewise, careless sneezing and coughing among butchers can lead to a contamination of the products. In addition, handling the carcasses and the money with the same unwashed hands could also be a good source of contamination. This unhygienic handling of the meats can affect the ultimate quality of the fresh product (Biswas *et al.*, 2011; Selvan *et al.*, 2007; Cohen *et al.*, 2006) and indicates that the lack of hygiene could be the major source of meat contamination at the main slaughter-house of Abidjan, Côte d'Ivoire. Salihu *et al.* (2010) indicated that the hygienic conditions are poor when foods are produced in non-industrial establishments, mainly because of the lack of required equipment for adequate processing.

The high load of bacteria on the samples analyzed could also be due to the long period of time the carcasses are displayed at the retail market and sold at high ambient temperature (32±4°C) on stalls without a chilling step. Contamination is uneven probably because of accidental contact with contaminated materials. The present findings were in accordance with the results of previous studies of Ukut et al. (2010). Rao et al. (2009) reported that most meats have a high water content corresponding to the water activity of approximately 0.99 which is suitable for a microbial growth.

Salmonella was isolated in 27% of the meat samples examined at the end of the marketing period, making these samples unfit for human consumption. Present finding agreed with those of Nisbet and Ziprin (2001) who indicated that the conditions of slaughter houses tend to allow the spread of Salmonella among carcasses with ranges upward from 21%. Jackson and Mcgowan (2001) stated that meat is considered spoiled when it is unfit for human consumption. At the end of the selling time (6: pm), 100% of beef carcasses sampled were of unacceptable quality as a result of faecal coliforms, Staphylococcus aureus or Salmonella, suggesting that it is better to buy the beef meat earlier in the morning. Results in this work were in accordance with those of Gormley et al. (2010) indicating that contamination with bacterial pathogens occurred earlier in the production chain. All the counts exceeded the established values in the microbiology criteria for beef meat. Storing the meat for sale in refrigerators (4-6°C) could preserve it and help avoid the drying of carcass surfaces and high microbial proliferation. Methods and developing technologies (superchilling, natural biopreservatives etc.,) for preserving fresh meat, proposed and investigated by Zhou et al. (2010) could also be applied to fresh meat products for high quality, safety, fresh appearance and an extended shelf life.

CONCLUSION

The results of this study showed the poor bacteriological quality of retail beef carcasses offered for sale in Côte d'Ivoire and indicated that the meats sold to the public are grossly contaminated with pathogenic bacteria (Salmonella, S. aureus, anaerobic sulphite reducers...) and viable source of various diseases. Some of these diseases could spread and reach epidemic level which could lead to serious public health problems. It seems therefore necessary to make the following recommendations from the findings of this study: 1) Meat handlers and sellers should be educated on the adverse effects of the lack of proper personal and environmental hygiene and sanitation; 2) Good manufacturing practices should be strictly adhered to by butchers and those selling the meat. the equipment must be washed properly before use; 3) Fresh meats should be well cooked before consumption.

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