

Applicability of Hazard Analysis and Critical Control Points (HACCP) System in Beef Processing Factories in Khartoum State

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Abstract

Since Sudan is applying to join WTO, complying with HACCP system in food manufacture is vital for both food safety and food trade. In the last two decades there are tremendous increments in processed meat industry in Sudan which require a unique food safety method to ensure products integrity. The goal of this cross-sectional, descriptive and analytical study is to evaluate the capabilities of meat factories in Khartoum State, so as to implement HACCP principles.

The current prerequisite programs (PRPs) of meat factories were assessed towards their adequacy for applying HACCP by constructed, standardized and scored checklist. Meat hygiene and safety competencies and HACCP awareness of meat processing workers were evaluated and 157 designed questionnaires were completed.

Microbiological investigation was conducted for eighty-six randomly collected samples of processed beef end product including; Mortadella, Burger, Sausage, Kofta and Minced meat to identify the most associated species of bacteria namely, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella spp.* and *Shigella spp.* However, apart from *Listeria monocytogenes*, all other targeted bacterial species were isolated from all types of processed meat. The quantitative bacterial species of total viable count showed high and critical levels. Sodium nitrites levels in 11% of beef sausage, 16.7% of beef kofta, and 22.2% minced beef and 27.8% of beef burger exceeded FAO acceptable limits.

In addition, forty samples of these products were analysed to determine the levels of added sodium nitrite salt (NaNO_2), and found to be exceeding the FAO acceptable limits in many samples. Furthermore; the final scores of assessment revealed that: the lowest mark was 45% and highest was 78%. Therefore, we did microbiological and chemical analysis, current hazards were identified, eleven major critical control points (CCPs) were recognized, control measures and corrective actions for each factory were suggested.

Based on the study results, there is a significant meat safety concern in studied factories. Out of nine factories for processed meat under study, only six could implement HACCP system after critical recommended adjustments. The main issue appeared from this study is that most of the risk factors are associated with the prerequisite programs of the HACCP system.

Keywords: HACCP; Beef; Meat; Processed beef; Pre requisite programs

Abbreviations: APC: Aerobic Plate Count; aw: Water Activity; BSE: Bovine Spongiform Encephalopathy (Mad Cow Disease); BSI: British standard Institution; CCPs: Critical control points; CFSAN: Center for Food Safety and Applied Nutrition (FDA); CFU: Colonies forming unit; CGMPs: Current Good Manufacturing Practices; CLs: Critical Limits (CLs); CODEX STAN: Codex Alimentarius Commission Official Food Standards; CP: Control Point; Directive: European Parliament and Council Directive; EU: European Union; FAO: United Nations Food and Agricultural Organization; FDA: Food and Drugs Administration; FDA: Food Safety and Inspection Service; FSIS: Food Safety and

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Inspection Service (USDA); GHP: Good Hygienic Practices; GMPs: Good Manufacturing Practices; HACCP: Hazard Analysis Critical Control Points; ICMSF: International Commission on Microbiological Specifications for Foods; ISO: International Organization for Standardization; MAP: Modified Atmosphere Packaging; NACMF: National Advisory Committee on Microbiological Criteria for Foods (USDA); NAS: National Academy of sciences; NASA: National Aeronautics and Space Administration (USA); PRPs: Prerequisite programmes; RA: Risk Assessment; SOPs: Standard operating procedures; USDA: United State Department of Agriculture; WHC: UNESCO World Heritage Centre; WHO: World Health Organization; MAP: Modified Atmosphere Packaging; ECSCVM: European Commission Scientific Committee on Veterinary Measures.

Introduction

Meat production is growing globally and it is estimated by FAO/WHO (2001) [1], that it will reach nearly 320 million tons by the year 2016. The rising demand for meat in developing countries is mainly as a result of the fast progression of societies becoming more urbanized. This is also apparent in Khartoum state where for the past few years; there has been a significant increase in the number of meat processing factories, as well as the assortment of products they produce. In 2009, when this study commenced, the numbers of meat processing factories in Khartoum state were nine as compared to year 2000 when there were only two factories. In addition, developing countries has also shown a continuous increase in meat consumption from an average of 10 kg per capita/year in the 1960s to 26 kg in the year 2000 and it is also estimated that it may reach upto 37 kg by the year 2030 [1].

Processed meat is very popular, particularly among younger population. Fast food products such as beef burgers or beef frankfurters are linked with urbanization [2]. Meat products if not handled properly, can lead to growth of food pathogens as well as hazardous microorganisms which represent a health risk to consumers.

Currently, application of HACCP system in food production in Sudan is not mandatory. Although there are many rules for food safety control, it is still relying on the food law of 1973, for that reason, meat industry in Sudan as well as the regulators are just relying on the conventional methods to ensure the safety of food. Hence, this reactive approach may lead to economic losses as well as delay in response to health hazards associated with food consumption [3]. Therefore, it is necessary to adopt a proactive approach to food safety. This can be achieved by establishing and implementing a food safety management system that will ensure that the meat industries are protected against legal action and that there is a continuous prevention of any potential occurrence of food safety related problems.

For the past three decades, the system known as Hazard Analysis Critical Control Point (HACCP) has been recommended by international and local organizations as well as United States Food and Drug Administration (FDA) and United Nation Food and Agricultural Organization (FAO) [4].

This system was developed in 1960s by USA Army Natick Laboratories, the National Aeronautics and Spaces Administration, and Pillsbury Company [5,6]. HACCP system is a scientific based system which identifies, evaluates and controls hazards which are significant for food safety. Currently, FAO/WHO (Codex) as well as World Trade Organization (WTO) has already adopted this HACCP system and soon it will be made a mandatory food trade tool globally. Thus, it is imperative that meat industry in Sudan also adopt and apply this scientific based system.

In reference to the various studies that assessed the contamination of meat, there is emphasis on the need of applying the HACCP system in food industries [7].

Any policy of preventive quality assurance should coincide with hazard analysis critical control point system (HACCP). It provides a more specific and critical approach to control the microbiological hazard in processed meat than that achievable by traditional inspection and quality control procedures. It involves identifying the hazards and their severity, determine points where hazard can be controlled specifying criteria which meet at these control points.

Therefore, as a part of a wider effort to develop a hygiene-promotion intervention for preventing meat contamination, HACCP principles were used to study contamination of processed beef production in Khartoum state Sudan. This is for the reason that, other authors have concluded in the past that, wherever the HACCP system was applied in food industry, it directly yielded positives results that not only benefited the producer, consumer and government but as well the quality of the product itself [8].

Practically, this study was conducted to investigate the control measures of contamination in beef industries by using the HACCP system's CCP concept and also to assess the maturity of common HACCP system prerequisites in meat processing factories so as to provide the current readiness for HACCP implementation purposes.

Materials and Methods

Settings

The study was done in all Khartoum State meat factories (nine factories) which are the only meat factories in Sudan.

Study design

In this cross-sectional, descriptive and analytical study, there were nine (9) meat-processing factories (Abu regella, Agwat, Al Arabi, AlGoussi, Amreen, Loli (2 factories), Maxim and Samar) evaluated according to applying HACCP system. For confidentiality purposes, these factories were coded in numbers throughout the study. The studied processed beef products were minced beef, beef sausage, beef burger and beef kofta.

Pre-requisite programmes assessment

To assess the status of the pre-requisite programmes (PRPs) and other related activities that could adversely affect meat safety and subsequent application of HACCP. A standardized and constructed checklist was developed and applied to all meat-processing factories, the checklist was divided into 15 elements, and further these elements were assigned a series of parameters to accurately describe the status of each assessed element. Each item under assessment was scored and points were assigned.

The checklists were completed during visits to each of the meat factories and the visits to each factory took place over a period of two to eight days.

Scoring system

A scoring system was used to distribute marks for each element of the PRPs according to the standard descriptive indicators. The comparison of facilities and prerequisite programmes (PRPs) status was achieved based on the scoring of the elements.

The numerical scoring system employed allowed for benchmarking, the setting of targets and the tracking performance and each element was divided into parameters, with each parameter having its own set of characteristics.

Each characteristic was rated depending on the significant risk of raw meat product being contaminated with the microorganisms or becoming contaminated with microorganisms or their toxins.

Determination of the risk and assigned scores

The most significant food safety parameters for each categorized element were determined during inspections of the meat processing facilities. The risk assessment was made based on the most significant food safety parameters.

The adequacies of the food safety parameters were assessed and a score was assigned for both written procedures relating to meat safety and actual practices observed during factory visits.

A score of zero (0%) was assigned when the factor posed a very high degree of risk to meat safety, whereas full marks (100%) were given when there was no risk to meat safety (Figure 1).

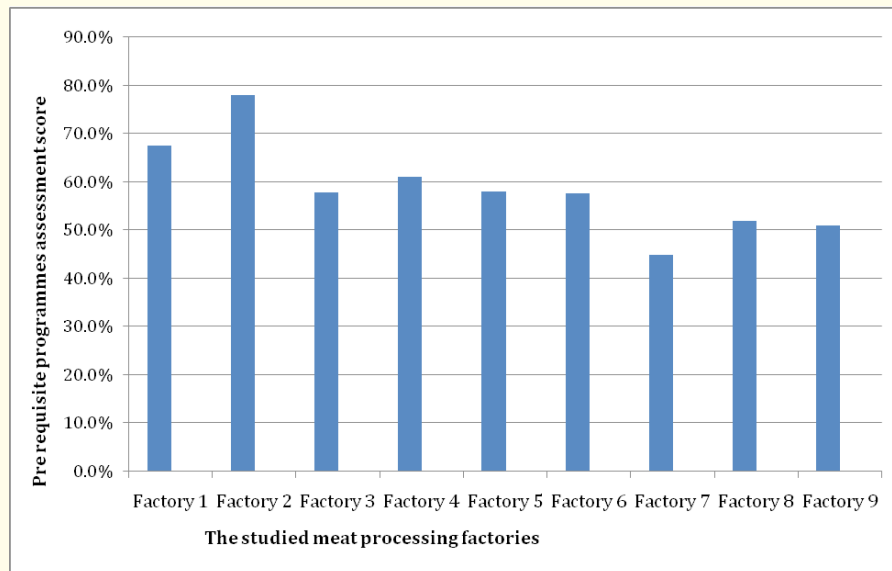


Figure 1: HACCP Pre-requisite programme qualification scores of meat processing factories.

Evaluation of the prerequisite programmes and adequacy

The total score of each meat factory was split into three categories depending on the risk of contamination and the possibility of cross-contamination.

Satisfactory: covered assessment scores of 75% or more.

Acceptable: covered assessment scores from 55% to 74%, where the HACCP plan, as well as the HACCP team is applicable.

Unsatisfactory: covered assessment scores below 55%, where HACCP system is not applicable.

Satisfactory' was defined as class I, 'acceptable' as class II, and 'unsatisfactory' as class III.

Bacterial hazards identification in processed meat

Hazard analysis approach was followed for investigating biological and chemical hazards in meat products in parallel with investigating the current status of each factory.

Eighty-six samples of various kinds of processed beef were collected and analyzed for seven species of bacteria associated with contamination of meat during processing, namely *E. coli*, *Staph aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella spp.* and *Shigella spp.*

The aerobic viable count was used to evaluate the total contamination of meats. Conventional methods using culturing in general and selective media followed by biochemical identification of isolates were employed.

Note: During the study, the advanced methods such as molecular biology were very rare and expensive in Sudan. Therefore, the standard conventional methods were used for bacterial identification.

Bacteriological sampling in processed meat

The microbiological testing was done quantitatively and qualitatively by applying the destructive method. Then, risk assessment (RA) was conducted using a structured approach based on the probability of an adverse health effects and the severity of that effects, significant to hazards in processed meat.

Most beef associated bacterial genera were analysed, evaluated based on the adverse health effects of particular importance in development of appropriate processed meat safety controls. These selected beef associated microbes were *E. coli*, *Staph aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella spp.* and *Shigella spp.*

Eighty-six samples of various kinds of processed beef were collected and analyzed for the seven species of aforementioned bacteria.

All bacteriological examinations of samples were done in the laboratory of microbiology, Faculty of Public & Environmental Health, University of Khartoum. Samples were collected over a period of fourteen months from various locations where the processed meat was distributed to the outlets and before displaying and storage of the products. The collection was done according to the visits of the meat plant facilities, when the facilities were evaluated, meat transporting trucks were followed until they reached the last point of distribution, and the samples were collected accordingly.

However, the hazard analysis critical control points (HACCP) approach was used to investigate progress and procedures that contribute to microbial contamination, growth, survival, and to identify point where control could be applied to prevent, eliminate these microbiological hazards or reduce to acceptable levels.

According to (HACCP) system the processed meat product samples should be collected from different stages of processed meat production, but end products were collected from sales points.

Note: Meat factories owners refused to collect the samples from the meat processing line (pre and post proposed each CCPs) as recommended by HACCP. Consequently, only end products samples were collected.

The collected samples kept inside the icebox under 8°C and delivered to the laboratory of microbiology at faculty of Public and Environmental health and promptly the samples were thawed and processed.

Processed meat samples preparation

Preparation of test samples for microbiological examination was done according to British Standard Institution (BSI), (1999). All apparatus and glassware that came in contact with diluents or samples, except apparatus that were supplied sterile were sterilized either by heating at 121°C to 175°C for not less than one hour in the oven or by heating at 121°C for not less than 20 minutes in an autoclave.

The samples reached the laboratory in frozen condition and were stored immediately in deep freezer and always examined on the same day. Samples were thawed in refrigerator. Defrosting was done at ambient temperature until the samples were completely thawed. This operation was not exceeding 2 to 3 hours as recommended by BSI [9].

Opening of packages were carried out under aseptic conditions to avoid external contamination. Sterile scissor was used to open the sealed packaging materials.

The samples were homogenized by mincing under aseptic conditions. Drips were put back before one second of blending process as indicated.

Conventional aerobic plate count method

As described by Matuian and Peeler [10] the plate count method procedures for analysis of frozen, chilled, precooked, or prepared foods were done. The above method was applied also before counting different targeted bacteria. Coliforms samples were cultured on MacConkey agar, *Staphylococcus aureus* on Baird Parker agar medium, *Bacillus cereus* on mannitol egg yolk phenol red polymyxin agar medium, *Clostridium perfringens* on cooked meat medium, *Salmonella* species on *Salmonella shigella* agar media and *Listeria monocytogenes* on palcam agar medium respectively. The plates were incubated at 37°C for 24 to 48 hours and colonies were determined accordingly as a viable colonies forming unit (CFU).

The detailed procedures that developed by the Association of Official Analytical Chemists (AOAC) [11] (1990) the standard methods for the examination of dairy products, (1993) and the American Public Health Association (APHA) [12,13], for calculating and reporting the APC has been followed.

Gram's stain method: Staining was performed as described by ISO 7218, (1996) and Barrow and Feltham [14]. The stained slides were evaluated microscopically (either with immersion or without immersion). Gram positive showed blue colour and Gram-negative red colour.

Spore stains method: The combinations of spore morphology were used for the identification of spores of *Clostridium perfringens* and *Bacillus cereus*. These organisms were divided into groups based on the shape, size and location of the spores within the vegetative cells. The microscope for stained spore obtained the organism determination.

Gram-positive Bacilli were encountered for further investigation for *Bacillus cereus* by conducting the tests of haemolysis on 5% Blood Agar, starch Hydrolysis, pigment, motility status, oxidase gas production, ability of the growth anaerobically, sugar fermenting (Glucose and Xylose), and the spore position status. *Clostridium perfringens* was cultured on cooked meat medium, then blood haemolysis status, Gelatinase, Lactose Egg Yolk Milk Agar, Motility status and spores position situation were conducted [15].

Gram-negative rod were determined microscopically for detection of *E. coli*, *Salmonella* and *Shigella* and followed by the tests of Indole, Methyl Red, Motility, Urease, Citrate, Kelligler's Ion Agar, Hydrogen Sulfide (H₂S), Gas Production, Oxidase, Oxidation Fermentation Media and Vogus Prosuaker.

Gram-positive cocci bacteria were determined microscopically for *Staphylococcus aureus* and gram-positive bacilli using tests of Catalase, Coagulase, Yellow pigment, anaerobic and aerobic mannitol utilization.

Determination of nitrite content in processed beef products

Chemical analysis was conducted for forty samples to determine the levels of added sodium nitrite in meat. These samples were taken from the same samples that subjected for bacteriological tests targeted that forty five samples should be studied. This target determined based on the samples collection visits which are 45.

As recommended by BSI, [16] and Mohamed., *et al.* [17]. The nitrite test was carried out immediately and/ or within 24 hours. For confirmation purposes, the test was duplicated for the entire samples. The test was applied only for the samples weight of more than 200 grams.

The samples were homogenized using meat mincer (passed it at least twice) and mixed.

Ten grams of the sample was weighted, and transferred into the conical flask. 5 ml of saturated borax solution and 100 ml of water at a temperature not below 70°C were added.

The flasks with contents were heated for 15 min in boiling water bath and repeatedly shaken.

The flask and its contents were allowed to cool at room temperature then 2 ml of solution (I) and 2 ml of solution (II) were added successively and mixed thoroughly after each addition. Solution (I) was prepared by dissolving of 106 gram of potassium ferrocyanide-trihydrate in one liter distilled water. Solution (II) was prepared by adding 220 gram of zinc acetate dehydrate and 30 ml glacial acetic acid to 1000 ml distilled water.

The flask contents then poured into a 200 ml one-mark volumetric flask, diluted to the mark with distilled water, and allowed to stand for 30 minutes at room temperature. The supernatant liquid was transferred carefully and filtered through the fluted filter paper to obtain a clear solution.

Test modification

In addition, of filtration using fluted filter, a centrifugation step for one minute was added, and the results showed highly clearance supernatant solution.

Colour measurement

Less than 25 ml of aliquot portion of the filtrate was transferred into a 100 ml one-mark volumetric flask-using pipette, and distilled water was added to obtain a volume of about 60 ml.

10 ml of solution (I) was added, followed by 6 ml of solution (III) that was prepared by dissolving of 445 ml of concentrated hydrochloric acid in 1000 ml of water. The flask was mixed and the solution was left for 5 minutes at room temperature in the dark.

2 ml of solution (II) was added, mixed and the solution left for 3 to 10 minutes at room temperature in the dark, then diluted to the mark with water.

The absorbance of the solution was measured at a wavelength of 538 nm in a 1 cm cell using the spectrophotometer.

Calculation

Nitrite content expressed in milligrams of sodium nitrite per kilogram, the calculation was done using the following formula:

$$N = 200 \frac{m1}{m0V}$$

Where, m_0 : is the mass, in grams, of the test portion;

m_1 : is the value given by the calibration curve for the mass of sodium nitrite in micrograms, corresponding to the absorbance of the test solution.

V : is the volume in milliliters, of the aliquot portion of the filtrate taken for the photometric determination.

Study results were displayed by use of excels and power point of Microsoft Corporation [18].

Meat Factories staff awareness evaluation

A questionnaire was designed and circulated among the workers to determine their level of awareness of meat safety and good hygienic practices. Items on the questionnaire included the fundamentals of processed meat contamination and cross contamination; preventive measures and food safety control activities. A total of 157 short questionnaires were completed on site during working hours.

Note: In addition to the general evaluation, an intensive analysis was done for each factory as an individual case study. Nine case studies were developed.

Results and Discussion

Bacterial Hazards Analysis in isolated samples

According to the standards of ICMSF [19] and Zeuthen., *et al.* [20] that; beef can be a source of foreign income and the standards of food can be implemented. Though the recognition of the bacterial significance at each CCP is highly required to develop the necessary preventive and controls measures to ensure the processed meats safety and quality.

To identify the impact of bacterial contamination of each step during processing the samples should be collected from the processing line and tested according to standards [19]. But, in this study, the managers and/or owners of all meat factories were refused to provide the meat and or non-meat ingredients samples from the process lines, therefore, the samples were collected from different sale points within the Khartoum State, where the products were distributed to the outlets, the samples were purchased from the factories trucks.

The study revealed that there was unusual high degree of contamination of targeted processed beef products in all different meat factories, which is greater than acceptable levels in all products. The number of microorganism was estimated and expressed as agreed by Heinz and Hautzinger; Ashton [2,21].

The range of a viable count of beef sausages product, minced beef, beef burger kofta products showed high levels of contamination in ranges more than 2.5×10^4 (cfu), which considered as a critical microbiological condition when compared with fresh meat [21,22].

Normal range is 10^3 to 10^4 organisms per gram as mentioned by Heinz and Hautzinger; Ashton [2,21] who stated that it is quite normal and unavoidable to find bacterial counts of viable count of the order of several thousand per cm^2 on meat surfaces in commercial slaughtering and meat handling. However, viable count numbers exceeding 100,000 per gram (10^5 per cm^2) on fresh meat are not acceptable. However, European Commission Scientific Committee on Veterinary Measures estimate the infectious dose range to be from 0.1×10^2 to 0.1×10^4 (cfu). Thus the detected range has clear implications on public health.

Six from seven targeted food borne illness bacteria (*E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella spp.* and *Shigella spp.*) were isolated from various kinds of beef products at different levels, which verified the presence of the bacteriological hazards as adopted by ICMSF [23] (Figure 2). It is worth to mention that, the severity of the biological hazards associated with meat ranged into three categories depending on the hazards effects. *Bacillus cereus*; *Clostridium perfringens*; and *Staphylococcus aureus* infection are considered as moderate, direct, limited spread, and death rarely occurs. *Salmonella enterica typhimurium*; *Escherichia coli*; *Shigella*; and *Listeria monocytogenes* infection are considered as moderate, direct, potentially extensive spread, death or serious sequence can occur. *Salmonella enterica subspecies Typhi* infection is considered as severe, and direct. *E. coli*, *Clostridium perfringens* and *Listeria monocytogenes* are meat-borne risks to human health [22].

The higher contamination level by *E. coli* was found in 5.6% of beef sausage product and the bacterium counts was reached more than 10^4 gram (Figure 2). Despite *E. coli* is usually used as an indicator of faecal contamination of food or water [24] however, certain strains are known to be pathogenic and some produce a toxin in the intestine that results in symptoms of abdominal pain and diarrhoea. The result showed that 50.0% of the isolated *E. coli* found grow and multiply for more than 10^2 colonies and 11.1% for more than 10^4 , this considered spoilage causes growth [25]. Certain strains are enteropathogenic and represent food born infection, and it can be used as an indicator organism of faecal contamination from raw material or during processing, the same method was followed in my previous study in AlKadaro abattoir [26].

The most unusual result was the presence of *E. Coli* in cooked (smoked) ready to eat mortadella product and the total viable count of *E. Coli* on mortadella product was found more than the infective dose (less than 10) microorganisms per gram was in 87.5%, and from 10^2 to less than 10^3 was in 12.5% of samples. This unexpected contamination might have been occurred either due to insufficient cooking temperature or after smoking.

In various levels, *Staphylococcus aureus* was isolated from the most processed beef products sampled (Figure 2). With regard to the potentiality of *Staphylococcus aureus* pathogen, it is commonly found on the skin and mucous membranes of humans and warm blooded animals [25,27]. Usually, the symptoms of *staphylococcal* intoxication appear when consuming food contains a toxin of less than 1.0 microgram. This toxin level is reached when *Staphylococcus aureus* populations exceeds 10^5 per gram. However, the study showed that the viable count of *S.aureus* was at the range of more than 10^4 was 11.1% in minced beef, 38.9% in beef burger, 27.8% in beef kofta, and 11.1% in beef sausage products samples. Such contamination level is considered hazardous since meat products might not be thermally treated to the degree that is capable of inactivating the heat resistance of enterotoxins which is commonly encountered in this bacterium species.

Foodborne illness caused by *S. aureus* enterotoxin is primarily a result of contamination by food handling personnel and is generally associated with temperature abuse of cooked products [28,29].

Due to the long heat treatment and the low a_w achieved by the extenders, mortadella product is consider shelf-stable products at moderate ambient temperature and may be stored without refrigeration [2]. However, the study showed that; 7.1% of *E.coli* and 14.3% of *Salmonella* were isolated from studied mortadella products this can be considered as a lack of cooking temperature during smoking treatment or mishandling during and after treatment (Figure 2). *Salmonella* contamination mostly indicated poor hygienic practices, which can enter the food from the raw materials or from meat processing handlers. It was estimated that 10^5 per gram of *Salmonella* bacteria are required in ingested food to cause Salmonellosis.

The growth of *Listeria monocytogenes* was not detected in all different beef processed products confirmed by repeating the tests (Figure 2). Inhibition of the growth of *Listeria monocytogenes* is probably due to the high concentration of residual sodium nitrite found in studied samples (5%), which was substantiated by USDA research indicating that sodium nitrite can help prevent the growth of *Listeria monocytogenes*, because of the water activity and pH attained during the initial lethality treatment by nitrite which may not support the growth of *L. Monocytogenes* during its refrigerated shelf life [30,31]. Furthermore, it is stated that the strong listericidal effects were observed when 0.3% sorbate was used in combination with 125 ppm sodium nitrite [32-34].

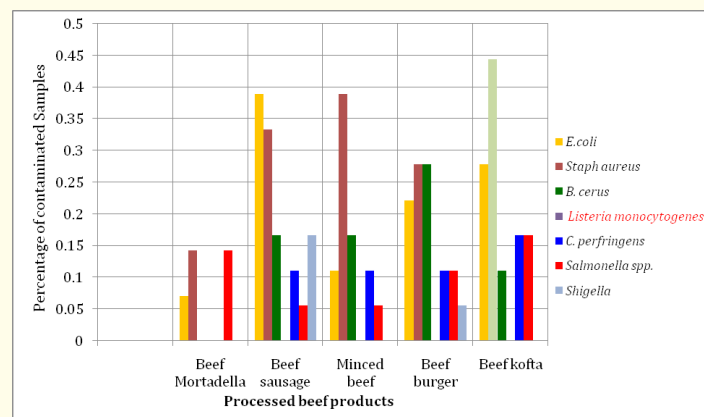


Figure 2: Processed beef products contamination by different bacterial species.

Bacterial hazards analysis in processed meat products

Beef sausage products were found heavily contaminated, the viable count (cfu) was 11.1% of the samples, and colonies were exceeding the range of more than 10^4 . Various food hazard bacteria were isolated, from the 18 samples of processed sausage beef; *E. Coli* (38.9 %), *B. Cereus* (16.7%), *C. Perfringens* (11.1%), *Salmonella spp.* (5.6%) and *Shigella spp* (16.7%). The intestines (natural casings) can contribute in this sausage contamination. Intestines should ideally be processed when still warm as they are easier to manipulate (cleaning, sliming, washing) and bacterial growth can still be contained and other good manufacturing practices are highly required before applying HACCP system [35].

From the beef processing line diagrams assessment, it was observed that frosting and defrosting practices could pose high risks of spore-forming bacteria, such as *Clostridium perfringens* and *Bacillus cereus*, these toxins will be treated even in further cooking. This step in the process can be considered a critical control point (CCP) and standard operating procedures (SOPs) should be established, adapted and monitored.

The study revealed that the viable count (cfu) in the range of more than 10^4 of *E. Coli* and *Staphylococcus aureus* was 5.6% and 11.1% respectively. The contamination could be introduced by raw meat, non-meat ingredients and surfaces in contact which meat including tables, meat hooks (at least parts in contact with meat), blades of knives, saws, cleavers and axes; and all parts of machinery in contact with meat, fat, sausage mixes and meat ingredients such as frozen meat cutter, grinder, meat mixer and tumbler, meat emulsifier, sausage stuffer, brine injector etc.

Comparing with other products, the minced meat contamination showed more than 10^4 (cfu) for isolated *E. Coli* (11.1 %), *B. Cereus* (16.7%), *C. Perfringens* (11.1%), and *Salmonella spp.* (5.6%) which is relatively lower than other product contamination percentage. In addition, *Shigella spp.* was not detected.

As observed that the contamination was most probably introduced by mishandling, through raw beef and/ or non-meat ingredients such as spices and vegetables. Also, in comparison with other products, minced beef showed low contamination as its considered as first or initial stage of processed beef product and not exposed to such long processing stages as other products, in addition of containing less additives i.e. meat and non-meat ingredients. Though groundbeef relatively protected from the cross contaminations because usually made from leaner, tougher and less desirable beef with small amount of additives [30].

The results exposed that the viable count (cfu) of isolated bacteria from beef burger was matched Doyle., *et al.* [36] statement, that *Salmonella*, *Staphylococcus aureus* and *Clostridium perfringens* are the main etiological agents associated with foodborne illnesses attributed to ground meat, including hamburger meat. Therefore the viable counts (cfu) of *E coli*, *Staph aureus*, *B cereus*, *C perfringens*, *Salmonella spp* and *Shigella spp* were 22.2%, 27.8%, 27.8%, 11.1%, 11.1% and 5.6% respectively.

The total count of the range of more than 10^4 was found 27.8% in 18 samples, which considered a very high contamination. Thus, in recent years, *E. coli* O157:H7 in ground beef products, hamburger in particular, have been implicated in a number of outbreaks of human illnesses in several countries [36-38]. Also, raw hamburger meat considered as a vehicle for outbreaks of human Salmonellosis in the United States [28,39]. Contamination of the raw beef combined with improper food-handling practices was found to be an important factor in a substantial proportion of the *Salmonella* cases [28]. Evidence suggests that the pathogen came from the slaughterhouses that supplied the raw material to the processing plant. Moreover, 25% of beef-related outbreaks in the USA, in the period from 1968 to 1977, were attributed to ground beef products, [28], whereas 21% of beef-related incidents of foodborne illness in Canada in 1983 were attributed to hamburger [40].

Ideally, the core temperature of frozen beef burger during cooking should reach 80°C to destroy food poisoning agents potentially present in the raw meat mixes such as *Listeria*, *Salmonella* and *E. Coli* [41].

Beef kofta product found also contain 27.8% *Staph aureus*, 11.1% *B. cereus*, 16.7% *C. Perfringens*, and 16.7% *Salmonella spp.* Mostly the source of these bacterial contamination could be from the beef cuts, non-meat ingredients, or/and meat processing handlers.

Chemical hazards analysis of processed meat products

Sodium nitrite is a dangerous, carcinogenic material [42] and is the only one chemical additive used in processed meat. However, as recommended that the maximum ingoing amount for processed meat products is normally up to 200 mg/kg of product [43]. On the other hand there are other agencies recommending 150 mg/kg [44-47]. Moreover, the residual sodium nitrite was determined specifically, in non-heat treated meat products is limited to amount of 100 mg/kg and could be 50 mg/kg [45] and the residual sodium nitrite in heat-treated meat products is 100 mg nitrite/kg [46]. To date, there is a reduction of residual nitrite in final products to 120 ppm in going nitrite has occurred in a number of countries [4,48,49].

Anyway, the study found that, 5% of the forty analyzed samples were exceeding the limit of more than 200 mg/kg (Figure 3). Thus, the results showed that; in 33.3% of mortadella; 66.6% of beef sausage; 77.8% beef burger; 66.6% minced meat; and 55.6% beef kofta, the residual level of sodium nitrate was found more than 125 mg/kg. Anyway, the amount of residual nitrite in the finished product should not exceed 125 ppm. [2].

Nitrites are toxic if used in quantities higher than recommended; therefore, caution should be used in their storage and use [4]. About 1 g or 14 mg/kg body weight the sodium nitrite is considered lethal dose to an adult human [50]. Epidemiological data indicated that the food poisoning occurred due to the accidental use of sodium nitrite and potassium arsenate [51]. The nitrite lethal oral dose for human beings is established as 33-250 mg nitrite/kg body weight [44].

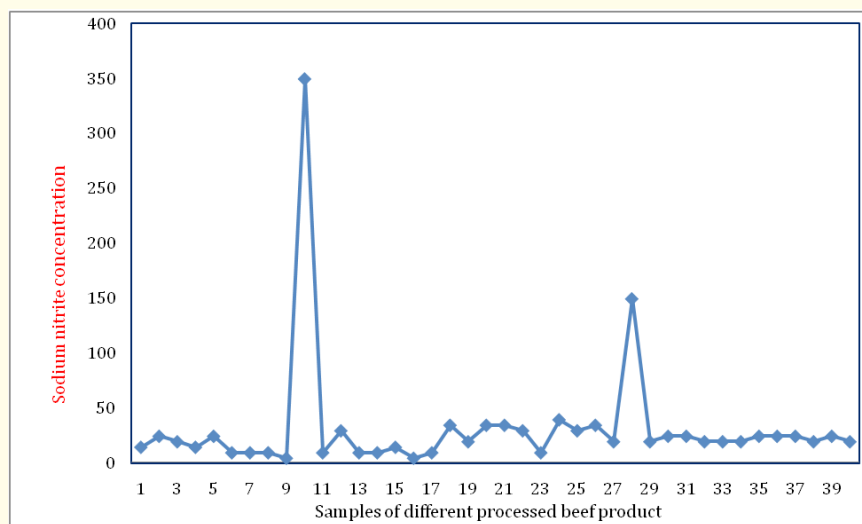


Figure 3: Sodium nitrite concentration in processed beef products.

Processed Meat Contamination Prevention and Control

The good manufacturing practices (GMP) and standard operational procedures (SOP) are fundamental programmes, to ensure the sanitary and food safety [52,53]. As stated by Orriss and Whitehead [54]; Taylor [55] Conter, *et al.* [56] these prerequisite programmes provide the basic conditions that are necessary for the production of safe, wholesome food. Though, the study was designed to assess these areas of HACCP success.

The study results found that the cold chains in the meat factories were inadequate to store the meat products, thus except factory 2, all factories were scored less than 77%. Most of the defects in the cold chain were related to defects in lighting equipment, absence of scheduled cleaning programme, lack of temperature checking activities, and deficiency in protection from the vermin. Definitely these issues will have impact on the produced meat safety and will affect applying of HACCP. As evidenced that, improper storage of processed beef has significant increase bacterial multiplication rates; particularly fresh sausages that are highly perishable products lead to fast microbial spoilage and oxidative rancidity [57,58].

To avoid this problem, the processed or under processing meat products should be stored immediately at the proper temperature, for maximum storage life of three days at +4°C or below. If the product at deep-frozen at -8°C, the storage life can be extended up to three months. But, one of the difficulties associated with frozen storage is the oxidative rancidity which can be solved by the store in vacuum bags [2,59-61]. However, it is worth to state that the study uncover that the packaging of some meat products was not been done under vacuum.

Anyway, and as recommended the entire meat processing factories refrigeration equipment used for storing or displaying must be capable of maintaining suitable temperatures and having sufficient storage capacity [2,62].

On the other hand, the results indicate insufficiency of non-meat ingredient storage which might cause contamination or cross contamination by microorganism. The results of dry ingredients and packaging material stores assessment of all factories were found less than 60%. Factory 1 was scored 71.2%; factory 2 was 57.5%; factory 3 was 61.6%; factory 4 was 60.3%, factory 5 was 67.1%; factory 6 was 61.6%; factory 7 was 53.4%; factory 8 was 54.8% and factory 9 was 60.3%. The main problems could be summarized in; deficiency of natural and/or artificial ventilation, no protection from vermin, absence of periodical cleaning of non-meat ingredients containers and the limited space between the shelves and walls. Before implementing HACCP a modifications are highly required to mitigate these problems. Thus, the dry food, fruit and vegetables stores should be well ventilated to maintain the required cool and dry conditions, the ventilation could be provided by either mechanical or natural ways [60].

Most of the meat processing factories in Khartoum State are located in poor -infrastructure industrial areas where sources of contamination such as dust, stagnant water, and congestion or redundant items were observed. The assessment of the factories surrounding contaminants scored less than 60%, in addition to the absence of effective pest-control activities, which could lead to processed meat contamination.

The sanitary design of all meat processing factories was found satisfactory, above 70%, except factory 5 was 42.3% which need to be improved. The evaluation was covered the meat factories sanitary design concerns such as cleaning and sanitation written procedures, checking of potable water quality and cleaning procedures for meat areas and meat contact surfaces.

Since improper cleaning and disinfecting were observed in all factories that there were no suitable disinfectants and no uses of hot water, which have an effects on meat process equipment hygiene. Sufficient sanitary design is essential to facilitate adequate cleaning and/or disinfection and therefore bacterial load reduction [63]. Portability of the equipment will enhance the cleaning and disinfection adequacy. Buildings structure materials must be suitable to allow appropriate cleaning [60,64,65]. Also, the number of hand washing facilities was found not enough and they are not supplied with hot/cold water, soap, drying facilities and dustbins, factory 1 was scored

36.7%; factory 2 was 76.7%; factory 3 was 6.7%; factory 4 was 36.7%, factory 5 was 46.7%; factory 6 was 40%; factory 7 was 16.7%; factory 8 was 0% and factory 9 was 23.3%. These results indicate clearly lack of hand washing and sanitary practices in general, which could be encountered as one of the factors that caused the high contamination by *E. coli*, *S. aureus* and other sanitary associated bacteria in sampled meat products. It is recommended by Codex [66] that, adequate number of wash hand basins must be available and suitably located and designated for cleaning hands. Hot and cold, running water, cleaning hands materials and hygienic drying. These basins must be separated from the hand washing facility.

Medical examination of meat handlers in all meat factories was carried out as prescribed by the controlling authority, as agreed by Gracey [67]. However, such medical examination tests cannot substitute the correct application of personal hygiene measures in preventing food borne diseases.

HACCP team formation and applying of the seven principles is required an effective training, in this regard, the result showed that none of the training programmes were conducted at all factories and 0% and 4.8% of trained workers were self-learning. This situation is discouraging applying HACCP or even other food safety systems continually disagreed by FAO/WHO [68] who stated that an employee training is mandatory when implementing HACCP system. Therefore, each meat-processing factory must have at least one employee who has successfully completed an approved HACCP training programme so as to form the HACCP team. Generally the lack of technically trained employees could have potential food safety problems. Furthermore, the regulatory authority should ensure that standardized training curriculum is met [69].

It appeared that 66% of the workers completed their secondary school and this education level qualifies them to utilize the food safety and HACCP training that is required to implement seven principles of HACCP including working instructions and procedures that outlines the task of employees to monitor each CCP [61,70].

In general, owners and operators of the meat factories may know about HACCP, but they have to hold competencies that could allow them to implement or maintain the HACCP principles [1,71]. Even with several years some factories will ignore the HACCP concept and Mortlock, *et al.* [72], which is a barrier to clear acceptance of the benefits of HACCP [73]. The most effective training is tailored to the trainees to suit their requirements and recognize psychological constraints on people [55,64,74].

At times, HACCP may need external expert support out of the factories to implement HACCP system at these factories [55,64,75].

From the study it was observed that most of the operators were hired on temporary basis and there are significant turning over; staff training can have long-term benefits, even with high staff turnover rates, as some staff will move to other businesses or sectors, and training can lead to increased awareness of food safety practices in the home [64,76].

Waste management in these factories was substandard including the waste collection equipments. The gained scored percentage was very low; most of them scored less than 46%. However, only three factories scored above 77%. In all factories, the waste areas were not protected from vermin. In most of the factories, the waste collection points were located improperly. The collection frequency was varied; some factories were disposing the waste by their own facilities and others through the municipality garbage trucks. The waste removal and waste collection points protection against accession of pests and against contamination is strongly recommended by codex Alimentarius [68]. Also waste containers must be of an appropriate construction, stored in sound condition, easy to clean and disinfect [2,68]. Therefore, when applying HACCP system at all factories, they should have waste management policy to protect the meat product contamination.

Furthermore, the study also showed very poor hygienic condition of toilets facilities and hand washbasins. Except factory 2 (88.20%), none of the factories exceeded 50%. This critical situation could negatively impact on the personal hygiene of meat processor and meat products, these areas could be considered as CCP when applying the HACCP system [61].

Following the meat processing flow charts and identified bacterial and chemical hazards revealed. The decision tree diagram approach of FDA (1999) was used to assess the risk associated with each points or condition in meat processing lines in the current meat factories in Khartoum State. Therefore, eleven critical control points (CCPs) were suggested to be recognized in these factories when implementing HACCP system, the CCPs were include; receiving raw meat, meat formulation, deboning, meat weighting, grinding; adding sodium nitrite, packaging, labelling, smoking temperature and cold stores temperature and final products transporting vehicles. Smoking (cooking), as critical control point can be applied only at seven factories, because factory 8 and factory 9 they did not have smoking house.

From study results of meat products analysed hazards categorizations of ADNOC (2004) [77] and PRPs assessment, the meat processing factories were classified into three class based on their capability and possibility of application of HACCP system, therefore class I or first class was found include factory 2, class II or second class: was included factory 1, factory 3, factory 4, factory 5 and factory 6 and class III third class, was found included factory 7, factory 8 and factory 9 (Figure 1). These results were obtained from excessive investigation and divided using scientific bases, which can be used as approach to identify the requirements of each group when applying HACCP and determination of the new similar factories.

The similar PRPs evolution result showed that HACCP plan can be possible applied at six factories (66.7%) of studied factories i.e. factory 1, 2, 3, 4, 5 and 6 (Figure 1). This was mostly based on identified hazards, assessed pre-requisite programmes, and evaluated personnel awareness, but prior to apply HACCP system an individual assessment for each factory is required to maintain the shortfalls that associated with individual PRP for proper implementation of HACCP system.

Conclusion

To produce safe and wholesome processed beef in Khartoum State and in light of the above investigations and findings obtained, HACCP system could be applied at six meat-processing factories out of nine (67%), however adequate controls need to be done in each factory in order to comply with HACCP system. The main problem appeared from this study is that most of the risk factors and shortfalls were associated with the HACCP prerequisites programmes since they are the base line of HACCP applications. This made the possibility of applying the HACCP plan is difficult. Although the HACCP and related food safety management systems application theoretically appear possible, as approved by the scoring system used, such application however could not be effective in controlling food safety hazards from processed meat in Khartoum State without correct fulfillment of prerequisites to HACCP system. The situation of applying HACCP system needs the full involvement and commitment of the food control authorities in the State as such prerequisites are fundamentals in food control regulations to facilitate proper and sustainable implementation of the HACCP system in meat product.

The study identified and evaluated the risks of bacteriological hazards from various species of bacteria isolated from beef i.e. *E. coli*, *Staph aureus*, *Bacillus cereus*, *Salmonella spp.*, *Shigella spp.* and *Clostridium perfringenes* from all studied processed beef. Sodium nitrite concentrations were detected and two samples (5%) of studied processed mortadella product were exceeding the recommended levels (more than 125 mg/kg). Factory 4 was 150 ppm and factory 4 was 350 ppm. Lack of awareness and training in employees with regard to meat hygiene and safety was noticed. From the assessed PRPs in all studied meat factories, the study categorized the factories into three groups.

Recommendations

National and state level control authorities shall play a role in regard to applying HACCP system in processed meat industry to provide a scientific approach to meat safety wholesomeness throughout the production, processing, and distribution of fresh beef, together with other quality assurance procedures. The success of a HACCP system depends on education and training management and employees for their role in producing safe foods. This should also include information on the control of food borne hazards related

to stages of food chain. It is important to recognize that the employees must first understand what HACCP is and then learn the skills necessary to make it function properly [78]. Specific training activities should include working instructions and procedures that outline the tasks of employees monitoring each CCP [79]. Establishment of in-house experts and consultants bodies to provide the necessary technical support for verification and accreditation of implementing HACCP system. Further studies of investigation on applying or implementing HACCP system are needed for each meat factory prior to developing HACCP system.

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