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HYGIENE STATUS OF SAUSAGES DEVELOPED WITH BOVINE BLOOD AS FAT REPLACER

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ABSTRACT

Blood is known to promote microbial growth when incorporated into food or used as a stand-alone component. However, due to its diverse applications, blood has been widely utilized in food production across the world. While blood can be a major source of microbial contamination in food, improper personnel hygiene and poor equipment handling during processing also contribute significantly to contamination. Hygiene and overall consumer health in product development are critical factors to consider. This project aimed to assess the suitability and safety of sausages produced using blood as a direct fat replacer. Fresh liquid blood was used to replace fat in sausage formulations at varying levels, with replacement proportions set at 0%, 5%, and 10%. The sausages were stored at 4°C and analyzed on days 0, 7, and 14 using standard analytical methods to evaluate microbial safety and quality. The results indicated a positive trend, demonstrating that the blood source was hygienic and well-handled during processing. Microbial counts throughout the 14-day storage period remained within the acceptable limits set by the Kenya Bureau of Standards (KEBS). Pathogenic bacteria, including *Salmonella spp.*, *Listeria monocytogenes*, *Campylobacter*, and *Escherichia coli*, were completely absent in all samples. The highest recorded microbial counts were 1.16 log10 cfu/g for *Staphylococcus aureus*, 1.47 log10 cfu/g for *Clostridium perfringens*, and 5.27 log10 cfu/g for total viable count (TVC). These values remained lower than the KEBS recommended limits of 6.0 log10 cfu/g for TVC, 2.0 log10 cfu/g for *Staphylococcus aureus*, and 2.0 log10 cfu/g for *Clostridium perfringens*. The findings of this study confirm that blood can effectively be used as a fat replacer in sausage production without compromising microbial safety, provided that strict hygiene and proper handling practices are maintained during processing. Ensuring clean production environments and proper handling procedures is essential to producing hygienic, high-quality sausages with blood as a fat substitute.

Key words: Sausage, Colony forming units, Hygiene, Contamination, Total viable count, Pathogen

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INTRODUCTION

Sausages are some of the meat products that possess a high potential for microbiological spoilage. Blood is one of the best sources of protein. It has in some cases been referred to as 'liquid protein'. All over the world, blood is produced after slaughter, and sadly majority of it goes to waste despite the fact that it has the potential to provide a higher and cheaper protein source than eggs. It has been estimated that China produces about 1,500,000 tons of blood yearly [1]. This has a protein content equivalent to that of 2,000,000 tons of meat or 2,500,000 tons of eggs [2].

On the other hand, blood is one of the most potent microbial broths used to proliferate microbial growth in laboratories for research institutions. Inclusion of blood as an ingredient, therefore, increases the vulnerability of microbial attack on the sausages. It is crucial to ensure that the blood used for the sausage or food product processing is fit for human consumption. While blood is not entirely the source of pathogens and harmful microbes, it cannot be ignored as an important source of the same. Improper handling and processing have been blamed for pathogenic invasion of blood-related products [3]. *Salmonella spp, Listeria monocytogenes, Clostridium perfrigens* and *Campylobacter* are usually of particular interest as they serve as indicators of food safety. They are dominant in meat products and related to disease outbreaks [4] *Listeria* and *Salmonella* are among the most lethal pathogens and are responsible for 1500 deaths annually in the USA [5]. *Escherichia coli* and *Staphylococcus aureus* are important indicators of faecal contamination of the blood and personal hygiene, respectively.

There are several ways that are typically employed to protect against the growth of bacteria in blood-laced products. Cold treatment remains the most common, coupled with HACCP system application in the processing areas[6]. Use of salts like nitrites and nitrates has also been a successful deterrent of bacterial growth in meat products. Bacterial growth inhibitors such as niacin have as well been used to reduce undesirable bacteria growth in meat products [7]. Food hygiene and safety is then clearly ensured through clean portion usage and operational cleanliness.

MATERIALS AND METHODS

The study was carried out at the Department of Human Nutrition and Dietetics, School of Medicine and Health Sciences, Kabarak University. The Analytical tests were carried out in the chemistry and microbiology laboratories.

Experimental design

The fat replacement ratios are shown in Table 1 expressed as percentages per kilogram. The meat and blood were obtained from a local slaughterhouse (Bahati

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slaughterhouse, Limuru) which possesses the required authorisation from local authorities about health and hygiene practices. Other ingredients were bought from authorised suppliers in Nairobi city.

Sausage production

The blood was carefully collected at the slitting stage. Sharp knives pre-sterilized in boiling water were used to sever the jugular of the cow. The slit cow was swiftly hoisted for bleeding with the head angled in such a way that it did not come into contact with the gushing blood. Collection was done immediately after hoisting in a cooled container; a large-mouthed sterile plastic container dipped in ice cubes. It was transported in cool boxes and stored below 4ºC to retain freshness and prevent microbial spoilage.

Fresh boneless semi-membranous beef from the thigh muscle immediately after slaughter was chopped into smaller pieces and minced with a 5mm sieve using a table top mincer. The minced meat was divided into five batches of equal quantities. The batches contained the standard sausage ingredients and fresh blood at different levels of substitution as shown in Table 2. Each treatment group was replicated three times. Additives and seasonings used in the formulations are shown in
Table 3.

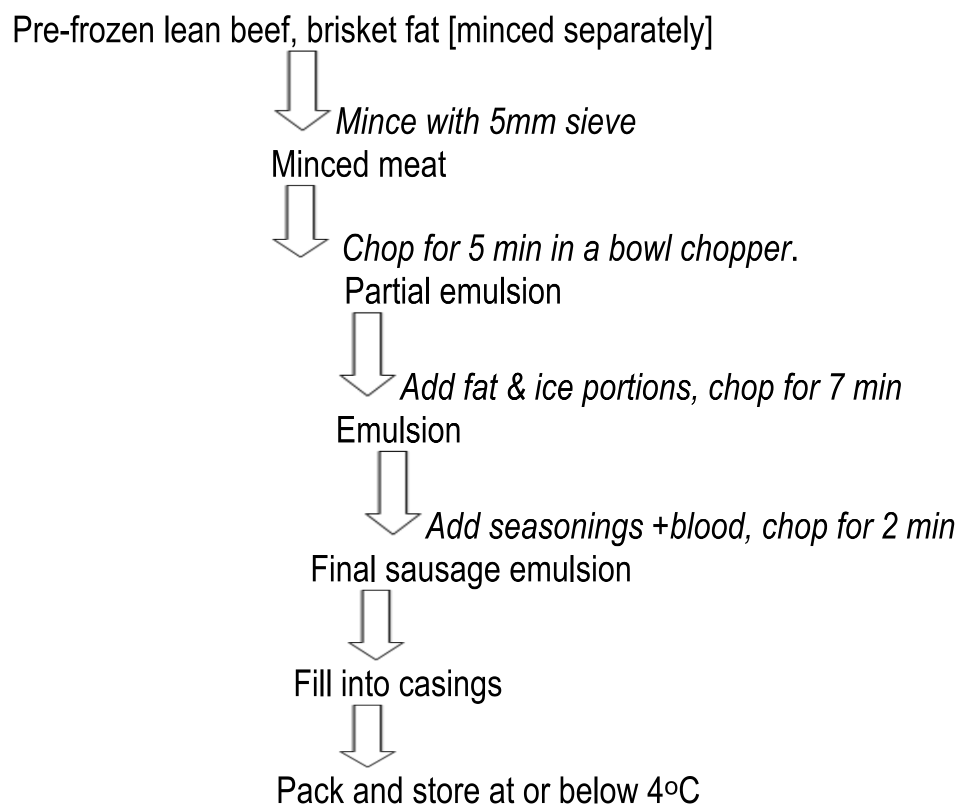

Figure 1: Sausage production process flow

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Analytical methods

The sausages were analysed for various microorganisms to determine the microbial profile so as to predict the general hygiene and overall keeping quality. For proper comparison with the sausages in the commercial market, the tests were carried out for a period of 14 days. This was aimed at accurate estimation of microbial shelf-life vis-a-vis the conventional sausages.

Tests were carried out in days zero, seven and fourteen on sausages stored in a refrigerator set at a constant 4ºC. Of interest were organisms that are relevant to meat safety problems. *Salmonella spp, Listeria monocytogens, Clostridium perfringens* and *Campylobacter* were of particular interest since they serve as indicators of food safety. These have been found to be dominant in meat products and related to disease outbreaks [8]. *Escherichia coli* was analyzed as a hygiene indicator, especially for faecal contamination. *Staphylococcus aureus* was used as an indicator of personal hygiene during processing while total viable count (TVC) were analyzed as an indicator of overall microbial quality of the sausages.

Determination of Total Viable Counts

ISO method 4833:2003 (ISO 2003) was used for the total viable count (TVC) enumeration. These are basically aerobic mesophilic bacteria. Duplicate plates containing plate count agar were used in the process. Incubation was done at 30±1ºC for 72±3 hours after which microbial counts were expressed as numbers of cfu/g of the sample.

Determination of *Escherichia coli*

Based on ISO method 16649-2:2001(ISO 2001), the *E. coli* was accordingly enumerated. 10g of sample was homogenized in 90 ml of peptone water. Decimal serial dilutions of the homogenized solution in sterile peptone water were prepared and plated in duplicate on the selective agar media. Blue-green colonies for *E. coli* were counted after 48 hours of incubation at 44ºC. The number of colony-forming units (CFU) of presumptive *E. coli* per gram of sample was calculated.

Determination of *Salmonella*

The ISO method 6579:2002 (ISO 2002) was used to enumerate the *salmonella* species. 25g of sample was blended in buffered peptone water and incubated at 37±1ºC for 18±2 hours. From pre-enrichment broth, the inocula were transferred to Rappaport-Vassiliadis broth and selenite cysteine broth and then incubated at 41.5±1ºC and 37±1ºC for 24 hours for selective enrichment. A loopful of the selective enrichment was streaked onto two solid selective media: Brilliant green agar (BGA) and xylose lysine desoxycholate agar (XLD). XLD agar was incubated

at 37±1°C and observed after 24±3 hours for a typical *Salmonella* transparent red halo and a black centre.

Determination of *Staphylococcus aureus*
EN ISO method 6888-1:1999 (ISO 1999) was used for the detection and enumeration of *Staphylococcus aureus.* In a sterile pipette, 0.1 ml of the appropriate sample test dilutions were transferred in duplicate onto the Baird Parker agar (BPA). The plates were then incubated at 35-37°C for 24±2 hours, then re-incubated for further 24±2 hours. Observation ensued for typical colonies appearing black or grey, shining and convex,1-1.5mm in diameter after 24 hours and 1.5-2.5mm after 48 hours of incubation, surrounded by a partially opaque zone. The coagulase-positive staphylococci were then expressed as cfu/g of sample.

Determination of *Listeria monocytogens*
Method 11290-01:2004 (ISO 2004) was used to enumerate the organism *Listeria monocytogen.* 225ml of Fraser broth with reduced concentration of selective agents otherwise known as half Fraser broth as selective enrichment media was blended with 25g of test sample. 0.1ml of the selective enrichment culture was transferred into 10ml of secondary enrichment media and incubated at 35°C for 48±3 hours. Plating was done using *Listeria* agar. Agar listeria incubation was done at 37°C for 24±3 hours. The carbohydrate utilization test was used to confirm the *Listeria monocytogens*. Typical *Listeria* colonies appear blue-green with an opaque halo on the *Listeria* agar.

Determination of *Campylobacter*
Enumeration was done using ISO method 10272-2006 (ISO 2006). 225 ml of liquid enrichment medium [Bolton broth] was added to 25g of the test sample. The contents were then incubated at 37°C for 4-6 hours and then 41.5°C for 44±4 hours after which incubation in a modified charcoal cefoperazone-deoxycholate agar (mCCD) at 41.5°C for 44±4 hours were done. For confirmation purposes, at least one suspected *Campylobacter* colony from each plate of selective media and a further four colonies if the first were negative were taken. Typical colonies are seen as greyish on mCCD agar, often having a metallic sheen and are flat and moist with a tendency to spread. The numbers of *Campylobacter* per gram of the sample were calculated from the number of colonies per plate.

Determination of *Clostridium perfringens*
Enumeration was done using the ISO method 7937:2004 (ISO 2004). 1 ml of appropriate sample dilutions was transferred and inoculated in a sterile pipette into empty petri dishes. 10ml of the sulphite-cycloserine agar (SC) which maintained at 44-47°C in the water bath was poured into the petri dishes and mixed well with the inoculum by gently rotating each dish. After the media solidification, a 10ml over

layer of the CS was added and allowed to solidify. The plates were then incubated under anaerobic conditions at 37°C for 20±2 hours. Plates representing successive dilutions and containing less than 150 colonies were selected and counted for the characteristic colonies presumptive *Clostridium perfringens* on each plate after the specified incubation period. Five selective colonies were selected and confirmed using Lactose sulphite medium test (LS) which has a very specific reaction to *Clostridium perfringens* when incubated at 46°C.

In this particular confirmatory test, each colony selected was inoculated into fluid thioglycolate media and then incubated under anaerobic conditions at 37°C for 24 hours. After incubation, 5 drops of the thioglycolate culture were transferred to the Lactose sulphite media with a sterile pipette. Incubation was then done aerobically at 46 for 24 hours in the water bath. Tubes containing lactose sulphite media were examined for the production of gas and the presence of a black colour [iron sulphate precipitate]. Durham tubes with over a quarter full of gas and tubes having the black precipitate were considered positive for the *Clostridium perfringens* which was expressed as cfu/g.

Data analysis

After enumeration, the counts were represented as colony forming units per gram (cfu/g). Microsoft excel was used to convert the figures into logarithmic version presented as $\log_{10}$.

RESULTS AND DISCUSSION

Table 4 shows the microbial profile for the sausages at different storage times and fat replacements.

Samonella spp, *Listeria monocytogens, Campylobacter* and *Escherichia coli* were absent in the sausages. The microbial profile of the sausages as per Kenya Bureau of Standards (KEBS) legal limits (KS 2455:2013, KS59-2:2013) requires that *Salmonella spp*, *Listeria Monocytogens* and *Campylobacter* be absent. *Listeria Monocytogens, Salmonella spp, Clostrifium perfringens and Campylobacter* are critical organisms that determine food safety status all around the world [8]. What's more, *Listeria Monocytogenes,* and *Salmonella spp* have been found to cause over 1500 fatalities in the USA through food contamination [9].

Several ways of microbial infestation control have been discussed in previous research for different meat products. The most effective of them has been to employ a HACCP system during production. However a prerequisite for the development of HACCP programmes is observance of good manufacturing practice [GMP] [10]. Cold treatment of meat products has been found to work against most organisms including *Samonella spp*, *Listeria Monocytogens,* and *Campylobacter* [11].

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Escherichia coli have been reported in meat products including sausages and blood-laced products. Improper or unhygienic food handling increases the chances of contamination by *Escherichia coli* [12]. Therefore, the absence of *Escherichia coli* in the sausages demonstrates hygienic handling as well as clean equipment and work area during the sausage processing. To further maintain personal hygiene, the food handlers wore clean overalls, hats and gloves. There were hand washing stations across the production areas as well as clean bathrooms.

Total viable count (TVC)
The TVC exhibited an increasing trend with the increase in blood content although the general count decreased as days passed by through-out the storage period. The highest value was for the 10% fat blood sausages on day zero at 5.27. On the other hand, there was a declining count throughout the storage period with the full fat sausages having the highest $\log_{10}$ cfu/g while the lowest was 3.97 $\log_{10}$ cfu/g on day 14 for the full-fat sausages. The TVC levels are well within the Kenyan safe limits for meat products which should not exceed 6.0 $\log_{10}$ cfu/g.

The high counts trend exhibited in the reducing level content of the sausages contradict with a previous study [13, 14] that found increasing microbial numbers with higher fat levels. This could be attributed to the highly nutritious blood in the sausages that make a good microbial broth as well as the high-water activity caused by the increasing blood in the sausages. Lack of nitrite pickling salt in the sausages that act as an anti-oxidant that prevent growth of bacteria could also explain the high number of organisms deep into the storage period [11].

Staphylococcus aureus
The *Staphylococcus aureus* count decreased with the increased fat replacement while also reducing with the prolonged storage period. The highest count for *Staphylococcus aureus* was 1.16 $\log_{10}$ cfu/g for full fat sausage on day zero while the lowest was 1.0 $\log_{10}$ cfu/g for the 10% fat replacement on day 14. The numbers fall within the Kenya Bureau of Standards [kebs], legal limits that set it at 2.0 $\log_{10}$ cfu/g. The decreasing numbers can be explained by the inactivation of the organisms by the low storage temperatures during storage. *Staphylococcus aureus* is usually used to indicate the efficacy of proper human handling of food during processing [15].

Clostridium Perfringens
The highest count for *Clostridium perfringens* was 1.47 $\log_{10}$ cfu/g for the control sausages on day zero while the lowest count was in day 14 for the 10% fat replacement at 1.25 $\log_{10}$ cfu/g. The counts were way below the lethal limit set out by KEBS at 4.0 $\log_{10}$ cfu/g. The decreasing trend for the organism can be accounted for by the low storage temperatures. *Clostridium perfringens* are important food

safety indicators that significantly feature in food borne disease outbreaks. The same organisms are actually linked to meat products bans by importing countries [8]

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The study proved that it is practically possible to use blood in sausage production without adversely affecting the microbial keeping quality. Proper blood collection, personal hygiene and hygienic handling during sausage production is paramount to ensure an acceptable general hygiene. It is important that other shelf life enhancing ways be established to increase the sausages longevity.

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Conflict of interest

The author declares no conflict of interest.

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Table 1: The fat, blood ratios in both gm and percentages

SAMPLE	BLOOD (%)	QUANTITY(g)	FAT (%)	QUANTITY(g)
1	0	0	20	200
2	5	50	15	150
3	10	100	10	100
4	15	150	5	50
5	20	200	0	0

Table 2: Ingredients in gm/kg for the sausages

SAMPLE	BEEF	BLOOD	FAT	ICE	WHEAT FLOUR	RUSK	CORN STARCH	ADDITIVES	SEASONINGS
1	500	0	200	180	60	40	20	19.8	3
2	500	50	150	180	60	40	20	19.8	3
3	500	100	100	180	60	40	20	19.8	3
Total	**1500**	**150**	**450**	**540**	**180**	**120**	**120**	**59.4**	**9**

Table 3: Typical additives and seasonings for a 1 kg sausage formulation

Additives	Quantity (gm.)	Seasonings	Quantity (gm.)
Common salt	16	White pepper	2
Sodium tripolyphosphate (STTP)	3	Nutmeg	0.3
Ascorbic acid	0.3	Mace ground	0.3
Monosodium glutamate [MSG]	0.5	Coriander & Ginger	0.4
Total	**19.8**	**Total**	**3**

Table 4: Microbial profile of the sausages during the storage showing staphylococcus aureus, Clostridium perfringens and TVC in log_{10}cfu/g

	DAY 0			DAY 7			DAY 14		
Fat:Blood [%]	**20:0**	**15:0**	**10:10**	**20:0**	**15:5**	**10:10**	**20:0**	**15:5**	**10:10**
Staphylococcus aureus	1.16	1.13	1.02	1.09	1.02	1.02	1.06	1.06	1.0
Clostridium perfringens	1.47	1.39	1.42	1.43	1.38	1.34	1.38	1.27	1.25
TVC	5.24	5.26	5.27	4.48	4.59	5.02	3.97	4.39	4.44

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