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Extraction, Evaluation and Application of Cashew Nutshell Extract for Ambient Preservation of Meats

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Abstract

Meat is a nutritious, protein-rich food which is highly perishable with a short shelf-life unless it is adequately preserved. There is the need to control meat spoilage using natural products rather than chemical preservatives. The preservative efficacy of cashew nutshell extract on beef and goat meat samples was investigated. Meat samples were cut and preserved with the cashew nutshell extract of four different concentrations (50 ppm, 100 ppm, 150 ppm and 200 ppm) for five storage days alongside the controls, without the addition of the extract under ambient condition. The yield of cashew nutshell extract after treatment was high (86.54%) at solute to solvent ratio 1:10. There was a statistically significant difference between meat samples preserved with cashew nutshell extract and the control group over the five storage days; beef ($P<0.05$) and goat meat ($P<0.05$). There was also a significant difference in the preservative efficacy of cashew nutshell extract between beef and goat meat up till the third storage day. The effect of concentration on the preserved meat samples also showed a dose response relationship; with highest and lowest 'Total Bacterial Count' (TBC) in beef at 50 ppm and 200 ppm respectively, while highest and lowest TBC were recorded at 100 ppm and 200 ppm respectively for goat meat. The acetone extract of cashew nutshell has the potential of preserving meats. Therefore, due to its natural antimicrobial food preservative properties, it will be very useful in meat industry.

L'Extraction, l'évaluation et l'application de l'extrait de noix de cajou pour la conservation ambiante des viandes

Résumé

La viande est un aliment nutritif et riche en protéines qui est hautement périssable avec une courte durée de conservation à moins que des méthodes de conservation ne soient utilisées. Il est nécessaire de contrôler la détérioration de la viande en utilisant des produits naturels plutôt que des conservateurs chimiques. L'efficacité de conservation de l'extrait de noix de cajou sur des échantillons de viande de bœuf et de chèvre a été étudiée. Des échantillons de viande ont été découpés et conservés avec l'extrait de noix de cajou de quatre concentrations différentes (50 ppm, 100 ppm, 150 ppm et 200 ppm) pendant cinq jours de stockage aux côtés

des témoins, sans ajout de l'extrait dans des conditions ambiantes. Le rendement en extrait de noix de cajou après traitement était élevé (86,54 %) à un rapport soluté/solvant de 1:10. Il y avait une différence statistiquement significative entre les échantillons de viande conservés avec de l'extrait de noix de cajou et le groupe témoin au cours des cinq jours de stockage ; viande de bœuf (P<0,05) et de chèvre (P<0,05). Il y avait également une différence significative dans l'efficacité de conservation de l'extrait de noix de cajou entre la viande de bœuf et de chèvre jusqu'au troisième jour de stockage. L'effet de la concentration sur les échantillons de viande conservée a également montré une relation dose-réponse ; avec une numération bactérienne totale (le TBC) la plus élevée et la plus basse dans le bœuf à 50 ppm et 200 ppm respectivement, tandis que le TBC le plus élevé et l plus bas ont été enregistrés à 100 ppm et 200 ppm respectivement pour la viande de chèvre. L'extrait d'acétone de noix de cajou a le potentiel de conserver les viandes. Par conséquent, en raison de ses propriétés antimicrobiennes naturelles de conservation des aliments, il sera très utile dans l'industrie de la viande.

Introduction

Meat is an excellent source of many nutrients, especially protein, B vitamins, iron and zinc. As a nutrient dense food, meat provides major nutritive contributions to diet relative to the amount of calories it contains. Red meat contains high biological value protein and important micronutrients that are needed for good health throughout life. It also contains a range of fats, including essential omega-3 poly unsaturated fats, sufficient water activity which supports the growth of both spoilage and pathogenic bacteria. Major spoilage organisms in raw meat are *Pseudomonas spp.,* others may include; *Shewanella, Brochothrix* and members of *enterobacteriaceae*. Growth of *yeasts* and *moulds* is essentially slow on fresh meat, compared to bacteria, therefore, they are not major components of spoilage flora (Dave & Ghaly, 2011; Doyle, 2007). The Food and Agricultural Organization (FAO) of the United Nations and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem and an important cause of reduced economic productivity (Käferstein, 2003).

Fresh meat has a shelf life of one day or less at ambient storage temperatures, 20-30°C (Mihretie, 2018). Spoilage is said to be a state of a particular food in which it is offensive to consumers' senses, usually caused by metabolites of contaminant microorganisms (Paulsen and Smulders, 2003). Meat spoilage is not always and totally evident and consumers would agree that gross discolouration, strong off-odours, and the development of slime would constitute the main qualitative criteria for meat rejection. As regards general spoilage, there is subjective judgment on the part of the consumer (Martinez *et al*., 2007; Beriain *et al*., 2009; Lou, 2009), which may be influenced by cultural and economic considerations and background as well as by the sensory acuity of the individual, and the intensity of the change (Nychas *et al*., 2008).

Meat available at retail outlets come through a long chain of slaughtering and transportation, where each step may pose a risk of microbial contamination. The sanitary conditions of abattoirs and its surrounding environment are major factors contributing to bacterial contamination of meat (Gill *et al*., 2000). Contaminations can be compounded during transportation, storage and handling of meat at butcher shops.

Meat preservation became necessary for transporting meat over long distances without spoiling of texture, colour and nutritional value after the development and rapid growth of super markets. The aims of preservation methods are to inhibit the microbial spoilage and to minimize the oxidation and enzymatic spoilage. Traditional

methods of meat preservation such as drying, smoking, brining, fermentation, refrigeration and canning have been replaced by new preservation techniques such as chemical, bio-preservatives and non-thermal techniques. Current meat preservation methods are broadly categorized into three methods which include: temperature control, water activity control and use of control through the use of chemical or bio-preservatives. However, consumers' concern over the possible adverse health effects of certain food preservatives resulted in increased pressure on manufacturers to remove chemically synthesized additives from foods and to provide more natural alternatives for the maintenance of food safety and shelf life (Abdulmumeen *et al.*, 2012). This is because annual per capita consumption increased to 26 kg in 2000 and will increase to 37 kg by 2030 as compared with 10 kg of 1960s (Heinz and Hautzinger, 2007). The present study was therefore designed to reveal the possibility of preserving fresh beef and goat meat using extracts from cashew nutshell, which has been implicated to contain antimicrobial properties (Gandhi *et al*., 2012; Mamidyala *et al*., 2013; Sharma *et al*., 2013,) against a wide range of bacterial species at ambient temperature.

Materials and Methods

Deboned meat samples; beef and goat meat were freshly collected in sterile polythene bags in an ice pack cooler from a slaughter house at Aleshinloye market, Ibadan, early in the morning, 45 minutes after slaughtering. The samples were transported immediately to the laboratory. The portions of beef and goat meat used in this experiment were from the thigh region of a matured cow and goat.

Extraction and Purification of Cashew Nutshell Extract (CNSE)

The extraction and purification of CNSE was performed as described by Idah *et al.*(2014), using acetone at a solute to solvent ratio of 1:10. The cashew nutshells used to obtain the CNSE were from Cocoa Research Institute of Nigeria (CRIN), Oyo State, Nigeria. The CNSE were extracted at the Institute of Agricultural Research and Training (IAR&T), Apata, Ibadan, Nigeria**.** Cashew nutshell extract (CNSE) were precipitated in the form of its Calcium salt. The Calcium salt was suspended in water and hydrochloric acid, and the resulting solution extracted with ethyl. The resulting organic fraction was then concentrated to obtain the refined CNSE.

Experimental Design

There were ten (10) treatments and three (3) storage times, with duplicates per treatment on each storage time, which amounted to a total of 60 observations. The cashew nutshell extract was added separately on the beef and goat meat in accordance with the treatments: (1) beef and goat meat without the addition of cashew nutshell extract (control), (2) beef and goat meat with addition of 50, 100, 150 and 200 ppm of cashew nutshell extract (CNSE). After preparation, the beef and goat meat were stored for 5 days, and analysed at days; 1, 3, and 5.

Addition of cashew nutshell extracts to beef and goat meat samples

Beef and goat meat samples weighing 10 ± 0.1 g were immersed separately in different concentrations of freshly prepared solutions of cashew nutshell extract in well labelled and transparent containers that were already in aseptic conditions. The samples were then stored for five (5) days under ambient conditions according to the experimental design.

Determination of microbial load in meats

Five grams of the beef and goat meat samples were weighed and aseptically taken into different sterile containers of 90 ml sterile normal saline. The meat samples were homogenized with sterile blender at 3000 rpm for 5-10 min. A 1mL aliquot of homogenate was transferred to a test tube containing 9 mL sterile distilled water to make 10^{-2} dilution and shaken well with vortex mixer. Serial dilutions up to 10^{-3} were prepared for the microbiological analysis according to the procedure of Ben-David and Davidson (2014). Diluted meat samples (beef and goat meat) in normal saline were spread onto plates and incubated at 37°C for 24hrs. The

microbiological quality and safety of the beef and goat meat were assessed on the basis of Total Bacterial Count (TBC), Total Coliform Count (TCC), using plate count agar and MacConkey agar (MCA), respectively.

Data Management and Analysis

The yields of the extracts were summarized in percentages. Descriptive statistics such as mean, and standard deviation were used to summarize the data on microbial load. Statistical Package for Social Sciences (SPSS) was used for statistical analysis at 5% probability for significance.

Results and Discussion

Comparison between the efficacies of cashew nutshell extract on micro-flora in beef and goat meat samples were analysed using the independent T-test. Also, the effect of cashew nutshell extract on beef and goat meat samples were analysed and compared using paired T-test. Comparison between the effect of concentration on beef and goat meat samples was analysed using the analysis of variance (ANOVA).

The yield of CNSE from the extraction and treatment process

The cashew nutshell extract yield from the treatment process was 90g which represents 86.54% yield of the total mass of starting material. Gandhi *et al*. (2012), also reported a similar yield of 80% for anacardic acid in their study on the effect of various solvents on extraction of cashew nut shell liquid (CNSL) and isolation of major phenolic constituents from extracted CNSL.

In the attempt to identify anacardic acid (the phenolic acid responsible for the antimicrobial properties) from the cashew nutshell extract, GC-MS did not identify anacardic acid as a major group and therefore was identified as essential oil. This is in consonance with the work of Patel *et al.* (2006) where the authors reported that GC-MS could not identify anacardic acid as a major group.

From the result of this study, acetone is a suitable extraction solvent for cashew nutshell if high yield is required at lesser solute to solvent ratio with support by the earlier reports of kumar *et al.* (2009), where various solvents were experimented using the soxhlet extraction process.

Microbiota in the meat samples

The presence of *Salmonella*, *Staphylococcus* and *Bacillus* in this study indicate contamination of the meats, possibly through air, water, soil and human carriers. Toxins liberated by certain strains of *Staphylococcus* and *Bacillus* are highly thermostable and can survive on cooking even at 100 °C for many hours according to the reports of Kibrom *et al.(*2019). The presence of spoilage type of microorganisms in the meat samples (as shown in Table 1) could be traced to mishandling of meat during transportation and unhygienic conditions of meat as earlier suggested by Rani *et al.* (2017) as well as Olusegun and Iniobong (2011). The ambient storage condition may also be the cause for increased number of microorganisms observed in the meat samples over the storage period. These bacteria are capable of producing maximum growth by consuming meat proteins and causing putrefaction and hydrolysis of fat. From the food safety point of view, this report is considered alarming with the knowledge that *gram negative* bacteria have been reported to account for approximately 69% of bacterial food-borne diseases, Kebede, *etal.* (2014).

Preservative efficacy of cashew nutshell extract against microbial flora in beef and goat meat

There was a statistical significant difference between beef preserved with CNSE and beef without CNSE over the storage days (P=0.00) as shown in Figs 1, 2 & 3. In a similar manner, the result of goat meat preserved with CNSE was statistically significant when compared with goat meat without CNSE on storage day 5 (P=0.00). However, statistical difference could not be determined for storage day 1 (for TBC and TCC), and also for TCC on storage day 3, but there was a linear reduction in the mean values of TBC and TCC in goat meat preserved with CNSE over the storage period as also observed in the beef preserved with CNSE.

Table 1: Bacterial micro flora in the meat samples

	Meat samples	
	Beef	**Goat meat**
Baccilus spp	+	+
Pseudomonas spp	+	+
Aeromonas spp	+	+
Enterobacter spp	+	+
Staphylococcus spp	+	+
Salmonella spp	+	+
E.coli	+	+
Flavobacterium spp	+	-
Clostridium spp	-	-
Listeria spp	-	-

"+" = present; "-" = not present

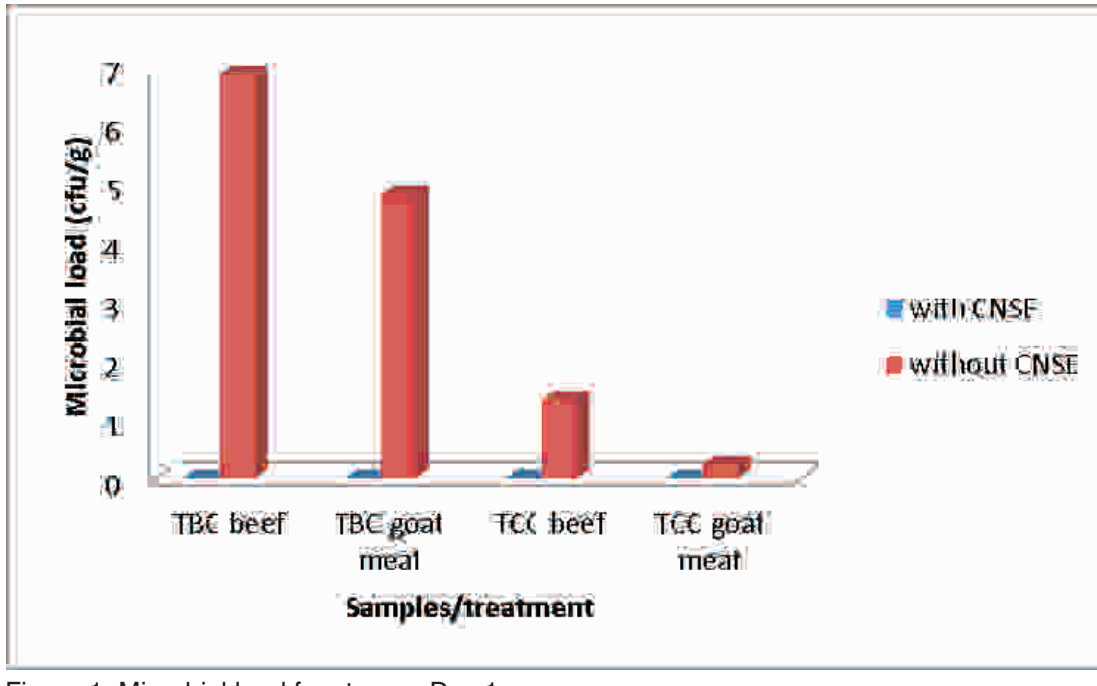

Figure 1: Microbial load for storage Day 1

The antibacterial activity of CNSL can be attributed to amphipathic phenolic compound; anacardic acid which enters into the membrane lipid bilayers, where various enzymes, especially components of energy converting systems such as electron transport chains (ETCs) and ATPases, are embedded. The amphipathic anacardic acids that enter into the lipid bilayers may disrupt the ETC and/or ATPases as surfactants.

Parasa *et al*. (2011), in their study evaluated the effect of acetone extract of Cashew (*Anacardium occidentale, L*.) nutsshell liquid against Methicillin resistant *Staphylococcus aureus* (MRSA) by minimum inhibitory concentration (MIC). These authors reported that crude extracts of cashew nutshell exhibited powerful invitro antibacterial activity against control and clinical isolates of methicillin resistant *Staphylococcus aureus*.

Saenaba *et al.* (2017), also reported the presence of anacardic acids to inhibit lipid synthesis of bacterial cells by inhibiting glycerol-3-phosphate dehydrogenase. Hollands *et al.* (2016), reported that

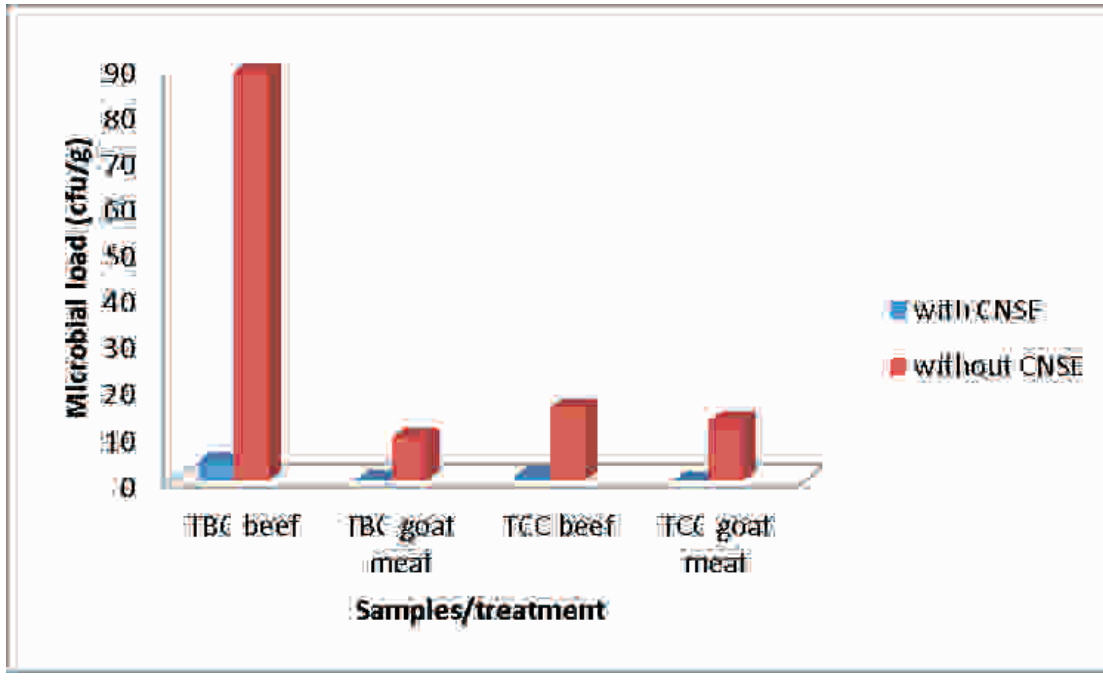

Figure 2: Microbial load for storage Day 3

chelation might also play a role in the antimicrobial activity of anacardic acids as it shows high selectivity towards Fe2+ and Cu2+ thereby reducing their bioavailability for bacteria. Phenolic compounds are secondary metabolites and one of the most widely occurring phytochemicals in plants that contribute to the sensory properties when added to food with antioxidant and antimicrobial characteristics that are useful in extending the shelf-life of food (Balasundram *et al*., 2006).

Comparison of the preservative efficacy of cashew nutshell extract against microbial flora in beef and goat meat

The difference between the preservative efficacies of cashew nutshell extract against microbial flora in beef and goat meat (as shown in Fig. 4 and Fig. 5), revealed a significantly higher preservative effect of CNSE in goat meat when compared with beef preserved with CNSE on storage day 3 only, as the mean TBC in beef was significantly higher than that of goat meat (P=0.008). However, there were no significant differences in microbial load (TCC) between beef preserved with CNSE and goat meat preserved with CNSE for all the storage days.

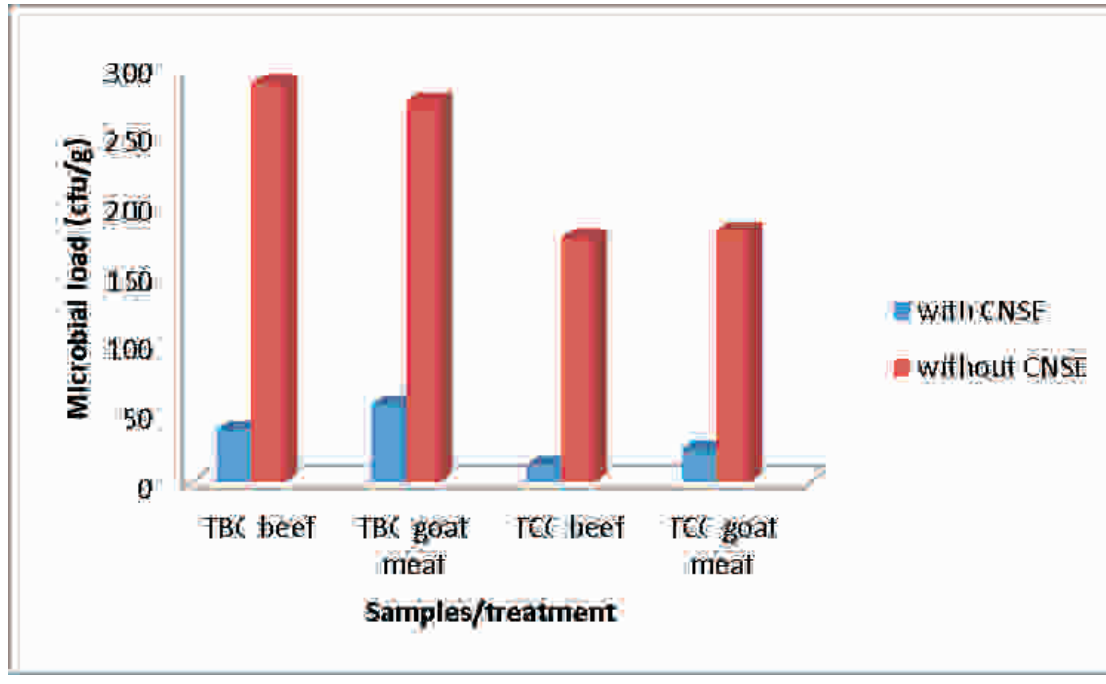

Figure 3: Microbial load for storage Day 5

Notably, there were increases in the mean TBC and TCC in both meat samples as the storage days increased. The preservative effect of the CNSE from the first day of storage up to the third storage day however decreased, after the fifth day.

This demonstrates that, in spite of the CNSE being able to inhibit the bacterial population (TBC) that grew, this effect only increased until 48 hours. This suggets thus suggesting that starting from this growth stage, microbial load in the meat was not controlled with the same efficiency.

This finding also agrees with earlier report by Ahmad *et al*. (2013), in their assessment that, the microbial load of raw meat at abattoirs and retail outlets in different areas of Lahore recorded a higher mean value for total bacterial count in beef when compared with goat meat from abattoir. Also in agreement with the present findings, is the report of Agwu and Chisom (2014), where they assessed the microbial quality of raw meat sold in Onitsha, Anambra State, Nigeria and reported a higher TBC in beef when compared with that of goat meat.

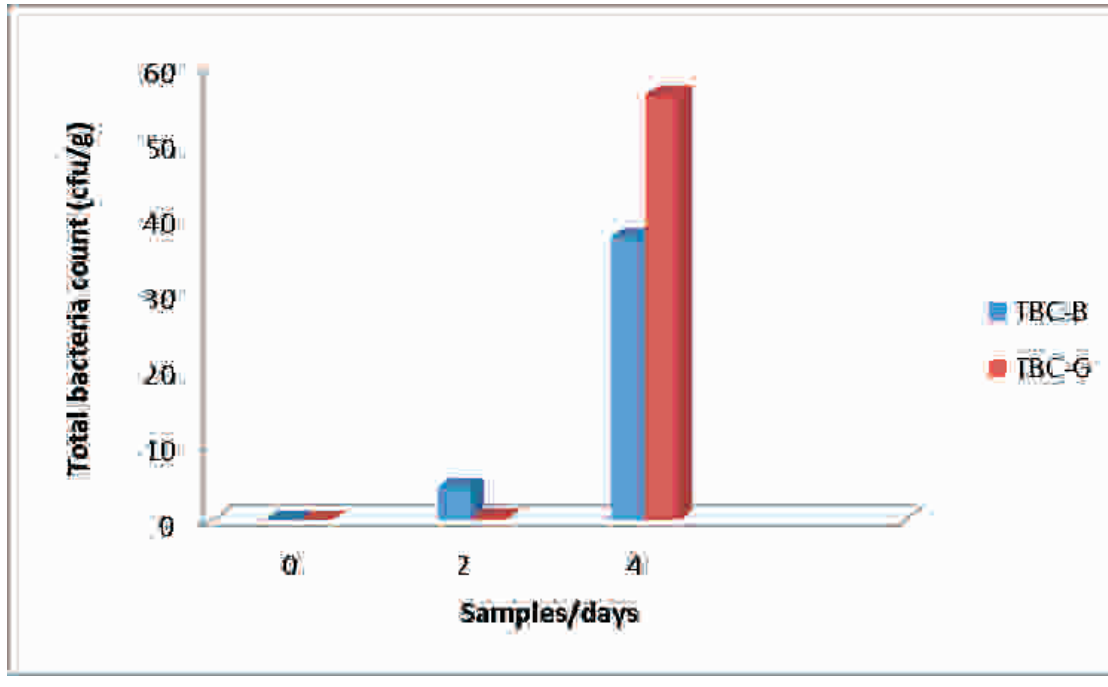

Figure 4: Difference in Total Bacterial Count (TBC) between beef and goat meat preserved with CNSE

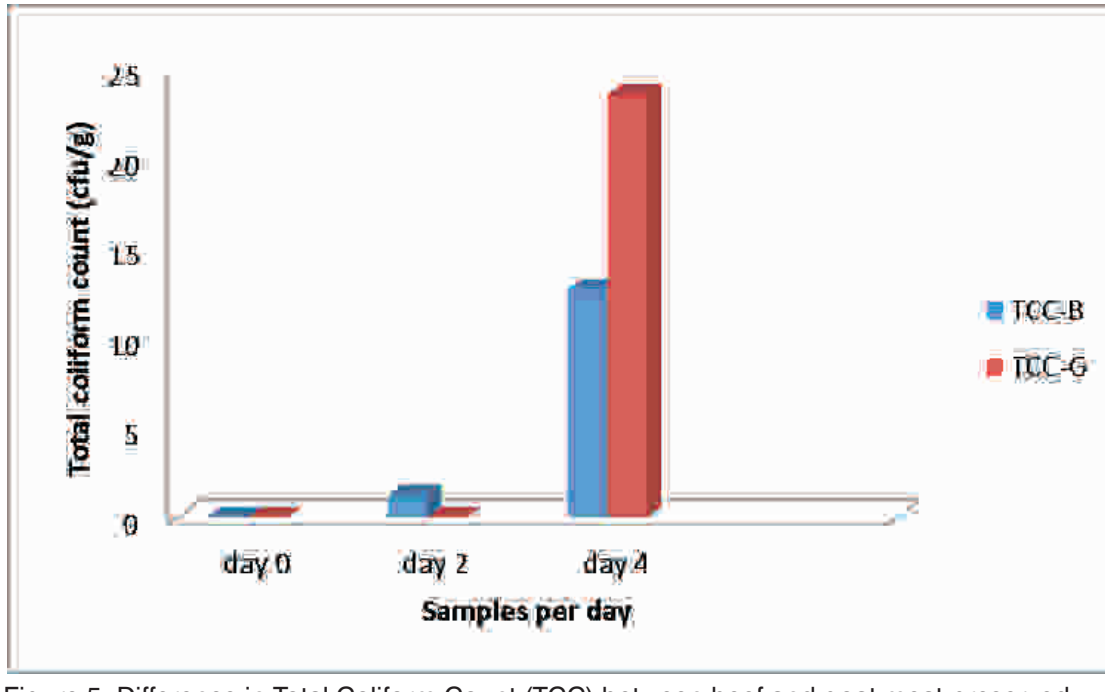

Figure 5: Difference in Total Coliform Count (TCC) between beef and goat meat preserved with CNSE

However, the findings from this study is in disagreement with the report of Chuku *et al*. (2016), on the bacteriological quality of fresh raw beef and goat meat retailed in Lafia metropolis, Nigeria, where the overall mean TBC for goat meat samples were found to be higher than that of

beef indicating that chevon was more contaminated than beef, even as the differences were not statistically significant. Likewise, in the findings of Antwi-Agyei and Maalekuu (2014), where they evaluated the level of microbial contamination of meat and fish products that were sold in two major markets in the Kumasi metropolis of the Ashanti Region in Ghana, there was also reported a significantly higher TBC for beef than goat meat.

The observed significant difference from this study may be attributed to the fact that beef was more contaminated than goat meat as shown in Fig. 4. It may also be due to the adequate presence of the needed nutrient substrates necessary for microbial flora in the meat samples, as beef is composed of more available nutrients than goat meat according to the reports of USDA (2001) on the comparative nutritional chart of meats. (Jay et al., 2005; Lado and Yousef, 2006; Lawrie and Ledward, 2006).

Effect of varied concentrations of cashew nutshell extract on microbial load in beef and goat meat preserved with cashew nutshell extract

In the determination of the effect of concentration on the preservative efficacy of cashew nutshell extract on beef and goat meat over the storage days (as shown in Fig. 6 & Fig. 7), there was no significant difference obtained in meat samples preserved at different concentrations over the storage days ($P>0.05$). However, linear reductions in TBC were observed with increased concentration of CNSE demonstrating a dose response relationship.

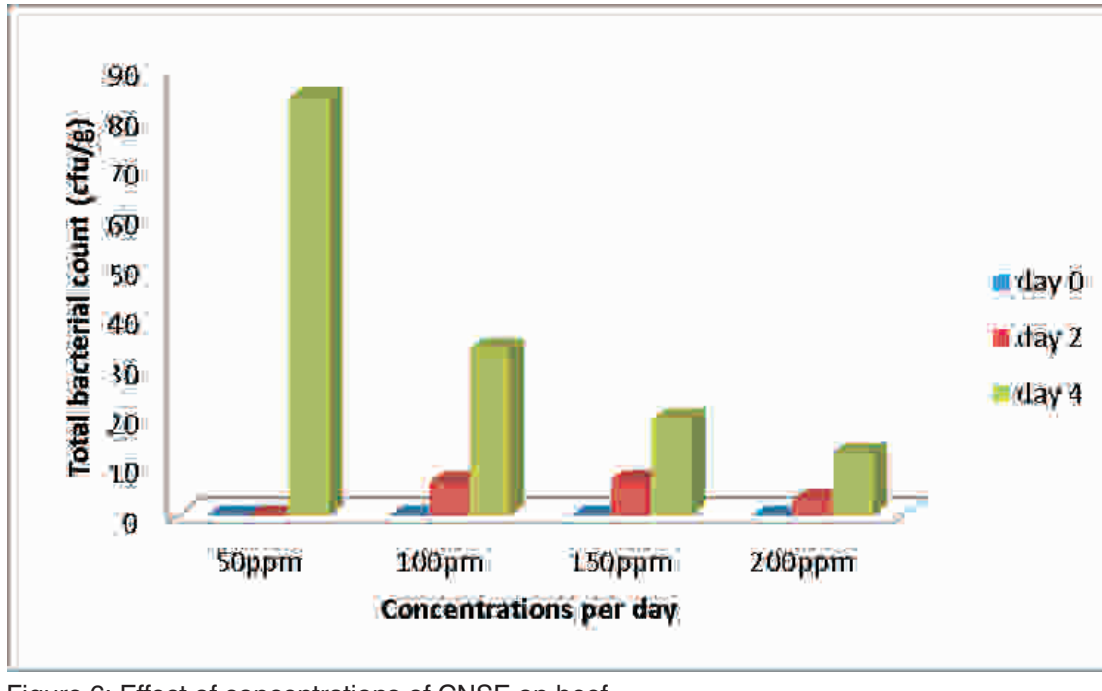

Figure 6: Effect of concentrations of CNSE on beef

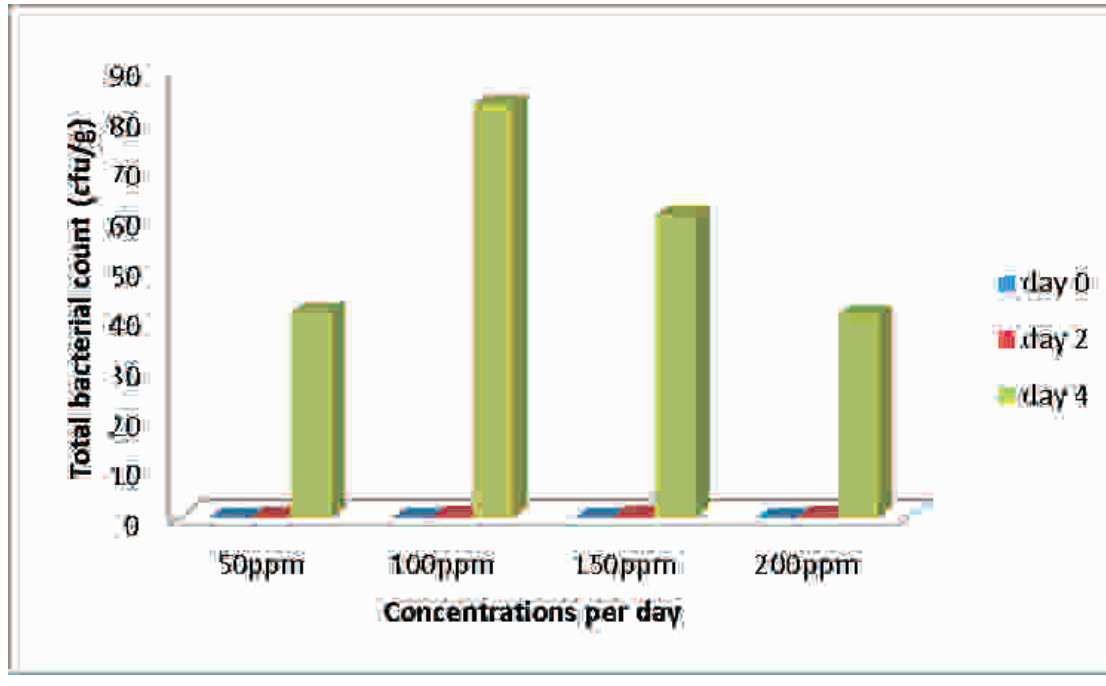

Figure 7: Effect of concentrations of CNSE on goat meat

Abreu *et al*. (2015), study on the addition of cashew nutshell extract as antioxidants in broiler chicken mortadella, reported that there was no significant difference in lipid oxidation during storage. Rather, there was a linear reduction in the thiobarbituric acid reactive substances (TBARS) values with increased dose of cashew nutshell extract in the mortadellas demonstrating dose-dependent relationship. Similar to the present findings, Qi & Zhou (2013) also evaluated the antioxidant effect of a lotus seed epicarp extract in growing concentrations in pork homogenates representative of Chinese Cantonese Sausage using TBARS. This present study also revealed that the lotus seed epicarp extract significantly retarded the level of lipid oxidation in a dose-dependent manner. Banerjee *et al*. (2012), in turn, using broccoli extract at concentrations from 10000 to 20000 ppm in the goat meat nuggets observed a decrease in TBARS values with higher levels of extract.

The possible modes of action of cashew nutshell extract as a phenolic derivative and antimicrobial agent have been previously reviewed (Davidson, 2001; Lopez-Malo *et al*., 2000; Marilen *et al*., 2018). The effect of phenolic compounds can be concentration-dependent such that, at low concentration, phenolic derivatives affect microorganism's enzyme activity, particularly those associated with energy production, while at high concentrations they cause protein denaturation (Chabi *et al.,* 2014).

Conclusion

The results of this study revealed that Cashew Nutshell Extract in the dose of 50, 100, 150 and 200 ppm contains natural antimicrobial preservative potential as regards its' addition to fresh beef and goat meat stored for up to 5 days under ambient condition (20 – 30 °C). Therefore, due to this preservative property, it is highly recommended for use in the meat industry.

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