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Evaluation of *Ziziphus abbysinica*, fruit paste extract as used by the pastoral community of West Pokot Kenya for the preservation of fresh pork sausages

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1 SUMMARY

The pastoralists from West Pokot district have used the fruit paste of *Ziziphus abyssinica* A. Rich (AZA), to process and preserve meat over a long period of Time. This study tests the effectiveness the herb to preserve meat using pork sausages. The effectiveness of AZA was tested by incorporating the aqueous extracts of the fruit paste of this herb, into samples of pork sausages stored at both 4ºC and 25ºC. Total viable count (TVC), *Staphylococcus aureus* counts, *Escherichia coli* plate counts, TVB-N and rancidity tests were undertaken over a period of 14 days. At 4ºC the samples preserved with AZA at the rate of 3.1mg/g of meat attained the total viable count (TVC) upper limit of 6 log cfu/g in about 10 days, and the TBARS value maintained below 0.9mgMA/kg during the same period. On the eleventh day at 4 ± 2°C the total volatile base nitrogen values of sausages treated with AZA herbs and sodium metabisulphite were 11.5 and 16.1mg/100g respectively. This may lead to the conclusion that AZA has higher antioxidant properties than sodium metabisulphite, while the latter has better antimicrobial properties than AZA. Further research on quantitative and qualitative analysis of the phytochemicals incorporating AZA should be developed.

1 INTRODUCTION

Background: Meat is one of the most nutritious but also most perishable foods. Unless it is preserved or stored under cool conditions, it rapidly deteriorates and becomes unfit and/or unsafe for human consumption. The pastoralists in West Pokot, Kenya, have applied indigenous knowledge and locally available tree species such as AZA, to preserve meat for hundreds of years (Mureithi, 1996). The pastoral communities of West Pokot also treat ailments like diarrhoea and various stomach infections using this herb. The Z. abyssinica or Angau (local name) belongs to the family Rhamnaceae that consists of small trees that are indigenous to tropical Africa and India. It is a Tree/shrub or climber of 1.8-8 m. Its bark is grey, deeply furrowed and branches are almost zigzag, with single or paired thorns of up to 12mm. It is found in wooded grassland, bushed grassland and along rivers. Its Cream pulp and outer skin are eaten. The pulp has a sweet to slightly bitter taste but edible portion is small. It is also used for construction, firewood, fodder, weapons, medicine, flavour enhancers, nutritional additives and appetizers (Uebelherr, 2005 and Sidigia et al., 1990).

In Eritrea, it is used for charcoal, medicine, bees forage and as live fence (Chikamai et al 2004). Its qualitative phytochemical constituents include alkaloids, saponins, flavonoids, reducing compounds, tannins sterols and steroids, and it is known to have antibacterial and antioxidant properties, which contribute greatly to its preservative properties (Nyaberi et al., 2010). Due to health concerns more people prefer consuming products preserved using herbal product other than the synthetic products in the market that are associated with side effects (Steinman, 2006; Murray et al., 1999 and Voravuthikunchai et al., 2004a). Inadequate scientific knowledge has often constituted a major constraint to the use of traditional herbal remedies (Krithika & Radhai, 2007). The fruit pulp of AZA is used by the people of West Pokot to preserve their meat. It is harvested around the end of December and January when ripe and red in colour. At this time most of the herbs have fruited, the rains have subsided, and therefore the concentration of metabolites is at the highest (Freitas and Glories 1999). The fruits are dried under the sun for about one week. The dried fruits are then crushed with stones to fine powder. The big particles of the seeds that don't get finely crushed are separated and the remaining powder dissolved in water to form a paste. Meat that has been cut in strips and dried either under the sun or smoked is immersed in the paste for about thirty minutes and then removed and left to dry under a shade or in the kitchen. This herb preserves meat for a about a year, it is preferred by the pastoralist of West Pokot because it does not leave its odour in the meat and the taste of meat remains the same. The pastoralists are also able to carry the meat with them as they look for pasture throughout the year. The aqueous extract of this fruit was incorporated in pork sausages to evaluate its potential to preserve pork sausages

2 MATERIALS AND METHODS

2.1 Study Site: This study was carried out in Chepareria division of West Pokot County, (Figure 1). According to the Arid and Semi-Arid Lands (ASAL) report of the Government of Kenya of 1998, West Pokot was one of the 20 districts of the Rift Valley province which covers an area of 9100km^2. The district is surrounded by the Turkana people in the north the Karamajong cluster of Uganda in the west, Transzoia in the south, Baringo and Marakwet in the east.

2.2 Materials

2.2.1 Collection of information and samples: During the collection of samples, introduction to the community was done with the assistance of the office of the District Agriculture Officer, Kapenguria. The information on the type of herbs used and the way preservation was undertaken, was obtained by personal observation and the focus for discussions guided by a questionnaire. All the respondents were from Chepareria Divisions of West Pokot District. Herbs collected consisted of one whose leaves, stem, fruits and flowers were intact for taxonomic identification at the East African Herbarium of the National Museums of Kenya alongside the fruits of herb for analysis in the Department of Food Science and Technology of Jomo Kenyatta University of Agriculture and Technology (JKUAT). Immediately after collection, the samples were stored in cool boxes while on transit to JKUAT Food Science Laboratory.

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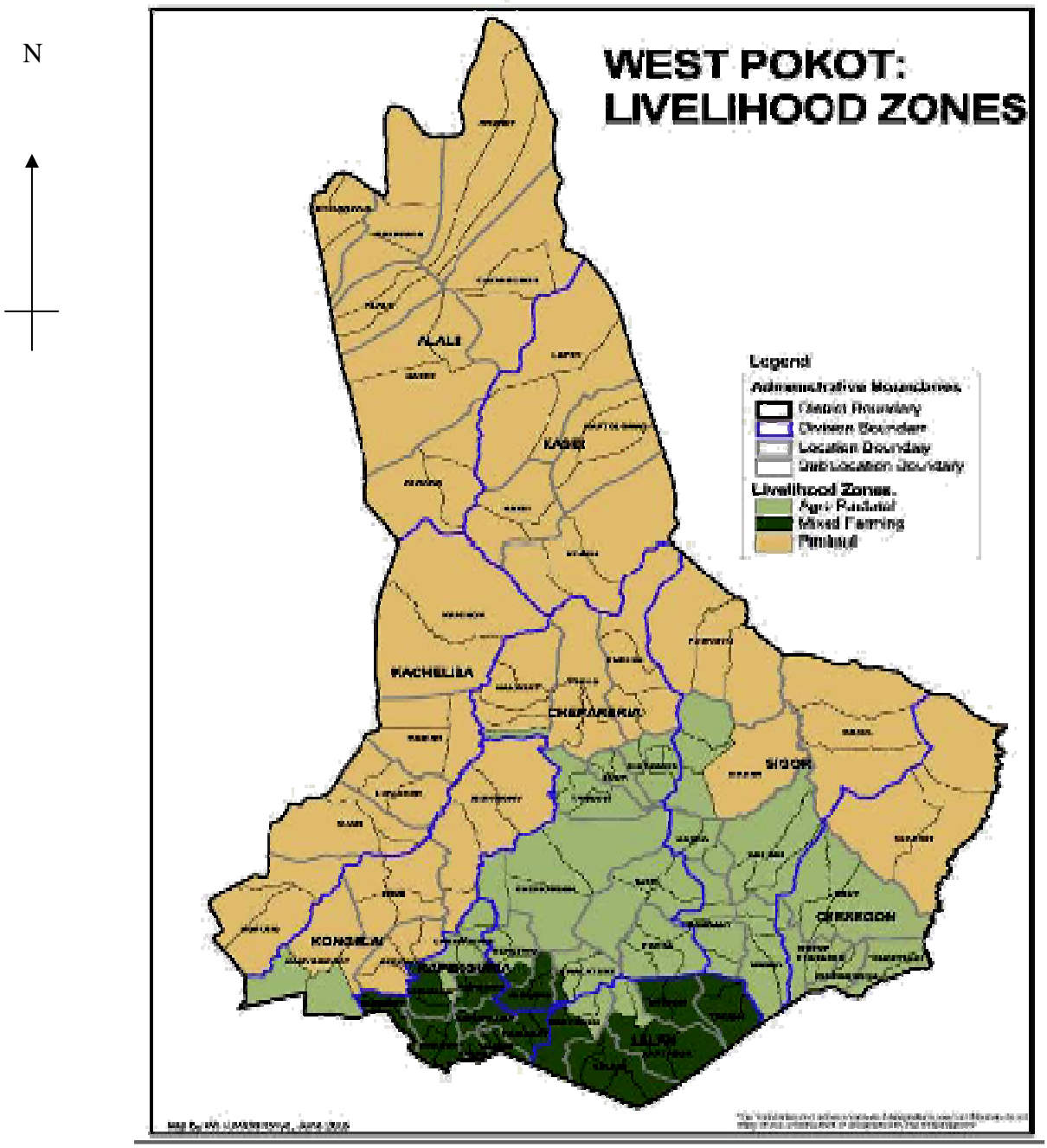

Figure 1: Map of West Pokot District showing the study site which includes Chepareria and Kongelai divisions (United Nations World Food Program (WFP), 2005)

2.3 Methods

2.3.1 Preparation of samples and extraction: A portion of the fresh herb of Ziziphus abbysinica (AZA) weighing 10kg (Plate 1) was gently cleaned using running tap water to remove soil then dried in controlled ambient temperatures of 25 ± 2°C in a room for six days. The fleshy part of the fruits of AZA were directly ground into moderately coarse powder using an electric grinder (model M10R Japan) and stored until needed for use (Onoruvwe and Olorunfemi, 1998)

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Plate 1: Herb collected from West Pokot for analysis (a) Angaun (AZA). (Photo by Nyaberi M. O. 2007)

A 500g portion of the dried herb sample was taken and cold extracted using deionized distilled water according to Bautista-Banos et al., (2003) and Regnier and Macheix, (1996)) with modifications. The herb sample was completely immersed in the solvent and shaken for 30 minutes to ensure sufficient contact with the solvent using a Kika Labortechnik Shaker, (Model KS 250 Basic, Staufen, Germany). The mixture was left to stand for four days in an enclosure at 25 ± 2°C. The mixture was then boiled for one hour, cooled, decanted and then centrifuged at 4,000rpm for 10 minutes at a temperature of 4°C using a Kokusan Centrifuge from Kokusan Corporation (Model 2000C, Tokyo Japan). The supernatant was filtered using a No. 1 Whatman filter paper and evaporated at below 80°C to dryness under a vacuum using a rotary evaporator (Model RE 100, Staffordshire, England). The extract obtained was put in a corked, light proof glass container and stored at 4°C till a time when it would be used (Mekbib et al., 2007).

2.4 Potential of herbs to preserve sausages: To ascertain that the herb actually has the capacity to preserve sausages, several tests were carried out. A 10kg leg part was obtained from freshly slaughtered chilled pork from the Pork Center Butchery in Thika (Kenya). It was washed, de-boned and stored at 4°C. The meat was divided into three batches of 1.8 kg each. Batch 1 was preserved with sodium metabisulphite at the rate of 2.6mg/g of meat; Batch 2 was preserved with AZA at the rate of 3.1mg/g of meat and Batch 3 was the control. Sausages were made by the method described by Njoroge et al 2008 with modifications (whereby the weights of the ingredients were varied due to the weight of the meat available but the ratios were maintained constant. The preservatives were also varied depending on their strength). From each batch sausages obtained were randomly separated into two groups. One group of the batch was stored at 4±2°C and the other at 25±2°C. All batches were analysed for rancidity (TBARS test) using the method of Tarladgis et al., (1960) as modified by Izumimoto et al., (1990), Total volatile base nitrogen TVB-N using the method suggested by the European Union (EU) Directive No. 95/149/EEC (1995). The proliferation of total viable counts (TVC), E. coli, S. aureus, yeast and moulds were done according to the AOAC method 966.23 (AOAC, 1995). The plates with colonies were counted and results expressed in log cfu/ml. All the tests were done over a period of 15 days at two days interval. The sensory analysis of the sausage was evaluated using a 9-point hedonic scale (Ihekoronye and Ngoddy, 1985). The whole procedure was repeated three times.

2.5 Statistical analysis: All the data was analysed for variance (ANOVA) using SAS computer program version 9.1. The comparison of the means, standard error and standard deviations at 5% level of significance were done using Duncan's multiple range tests (Steel and Torrie, 1980).

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3 RESULTS AND DISCUSSION

The plant was positively identified at the National Museums of Kenya in 2007 as *Ziziphus abyssinica* A. Rich in the class Rhamnaceae. Locally it is known as Angau, while in this paper it is abbreviated as AZA.

3.1 Total viable count (TVC): The total viable counts in all sausage samples stored at 4 and 25ºC ± 2 increased significantly (P<0.05) (Figure 2 A+B). The British Meat Processors Association (BMPA, 2006) recommends a maximum upper limit of 6-log cfu/g (Brewer et al., 1992). At 4ºC the samples preserved with AZA at the rate of 3.1mg/g of meat attained the upper limit in about 10 days; those preserved with sodium metabisulphite at the rate of 2.6mg/g of meat attained the upper limit in about 15 days, while the control attained the upper limit in about 5 days. This shows that the herb AZA had the potential to control the growth of micro-organisms though not as effectively as the synthetic preservative sodium metabisulphite. At 25 ± 2°C, by the second day all the sausages had surpassed the upper limit (Figure 2B). The counts were high and considered objectionable (El – Marrakchi et al. 1990; Ryder et al. 1984). There was no significant difference in the growth of micro-organism among the sample sausages and therefore the preservatives AZA and sodium metabisulphite had minimal preservative capacity when stored at room temperature, but unlike the control the other sausages did not produce a foul smell in the second day. This therefore indicated that at room temperature the preservatives may be reducing the development of rancidity.

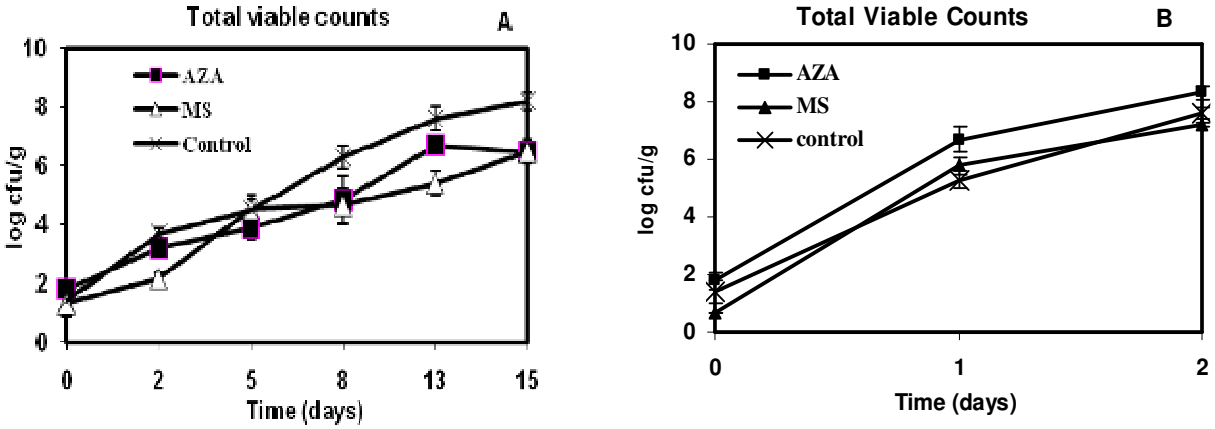

Figure 2: Total viable counts of sausages treated with herb extract AZA, sodium metabisulphite and a control at (A); 4°C, (B); 25°C.

3.2 E. coli and S. aureus counts: During storage at 4 ± 2°C, growth of E. coli and S. aureus was detected and increased constantly until the thirteenth day when the counts started to decrease (Figure 3). There were no significant differences (P<0.05) between the growth of E. coli and S. aureus in either sausages preserved with AZA or sodium metabisulphite. The 13th day recorded the highest count of 3.1 log cfu/g for AZA, 2.0 log cfu/g for sodium metabisulphite and 2.9 cfu/g for the control. According to the BMPA, the micro-organisms were within the acceptable limit of below 4.0 log cfu/g (Domańska and Różańska, 2002), (Figure 3). The fact that E. coli and S. aureus growth at 4°C remained within the acceptable limits including the control, ruled out the importance of using preservatives to control their growth at 4°C. At 25 ± 2°C, the growth of E. coli and S. aureus in the sausages preserved remained within acceptable limits of 4.0 cfu/g only up to the second day. Therefore, this may indicate that if meat sausages were hygienically handled and immediately stored at 4 ± 2°C then introduction of preservatives with the intention of controlling E. coli and S. aureus would be unnecessary. After the 13th day, the counts of E. coli and S. aureus fall, yet the sausages continue to deteriorate in taste and texture. This indicates that there may be other factors contributing to spoilage of these sausages other than the two common spoilage bacteria E. coli and S. aureus. Other micro-organisms that have been known to cause spoilage

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in sausages include Pseudomonades and Salmonella spoilage bacteria (Fang et al 2006**).**

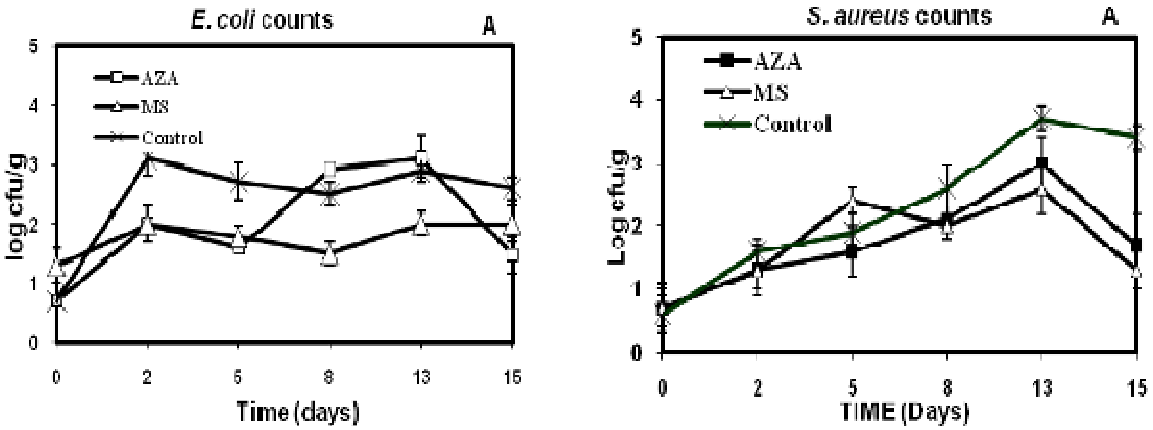

Figure 3: Growth of E. coli and S. aureus in sausages treated with herb extract AZA, sodium metabisulphite and control at (A); 4°C

3.3 Yeast and molds count: At 4 ± 2°C, the sausages preserved with sodium metabisulphite had significant (P<0.05) inhibitory effect against yeast and moulds compared to AZA and the control (Figure 5A). As from the 1st to the 13th day the sausages preserved with sodium metabisulphite had the lower counts of 2.8cfu/g compared to those preserved with AZA with 6.7cfu/g on the 8th day which was equal to the control. This therefore, shows that sodium metabisulphite is better than AZA in inhibiting the growth of yeast and moulds. It also indicates that AZA has the capacity to inhibit the growth of the yeast and moulds and could be used to inhibit the growth of yeast and moulds in pork sausages for six day at 4 ± 2°C (Figure 5).

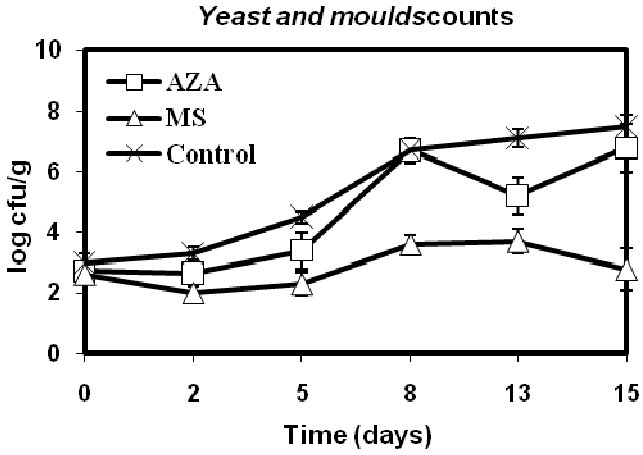

Figure 4: Growth of yeast and moulds in sausages treated with herb extracts and sodium metabisulphite at 4°C

3.4 Evaluation of rancidity (TBARS) in sausages treated with herbs: Freshly prepared sausages had a thiobarbituric acid reactive substance (TBARS) value of 0.1mg MA/kg (MA) which was within the recommended limits of 0.3mg MA/kg. Therefore, sausages stored at 4 ± 2°C showed a significant (P<0.05) increase in TBARS values across treatments (Figure 6). Samples treated with sodium metabisulphite attained 1.53 mg MA/kg on the fifteenth day while those treated with AZA had TBARS value of about 0.9mgMA/kg during the same period. This implies that AZA had greater

antioxidant activity compared to sodium metabisulphite (Figure 6). Research further indicates sausages treated with commercial preservatives BHA/BHT had TBARS value of 1.19mg MA/kg on the 14th day (Kawole et al 1996; Lee and Decker 1997). This value is higher than that of AZA in the 15th day at 0.9mg MA/kg and shows that AZA may have better antioxidant activity than the commercial antioxidants commonly used in meat products such as BHA/BHT.

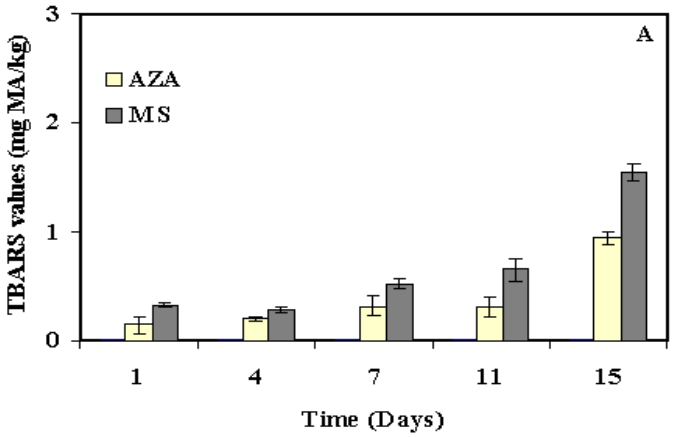

Figure 5: TBARS values at 4°C (A) for sausages preserved with AZA herb extracts and sodium metabisulphite.

3.5 Total volatile base nitrogen (TVB-N): The recommended upper limit for total volatile base nitrogen (TVBN) in pork sausages is 25 mg/100g (Lannelongue et al. 1982). On the eleventh day at 4 ± 2°C the total volatile base nitrogen values of sausages treated with AZA herbs and sodium metabisulphite were 11.5 and 16.1mg/100g respectively. This indicates that sausages treated with mild extracts of AZA could retain acceptable quality until the eleventh day. Total volatile base nitrogen is produced because of microbial activity and the low values obtained concurred with earlier findings of low total counts (>5.5 log cfu/g) for all treated sausages (Figure 2). On the fifteenth day, all the sausages treated with herbs extracts and sodium metabisulphite were slightly above the recommended upper limit. Samples treated with AZA had higher TVBN value than those treated with sodium metabisulphite, 33.3 and 30.3 mg/100g respectively on the fifteenth day. This agrees with observations of TVC recorded at 6.5log cfu/g just above the recommended upper limit at which microbial spoilage was observed to be highest. The TVBN values are normally used for rapid assessment of spoilage and are highly correlated with increase in microbial growth.

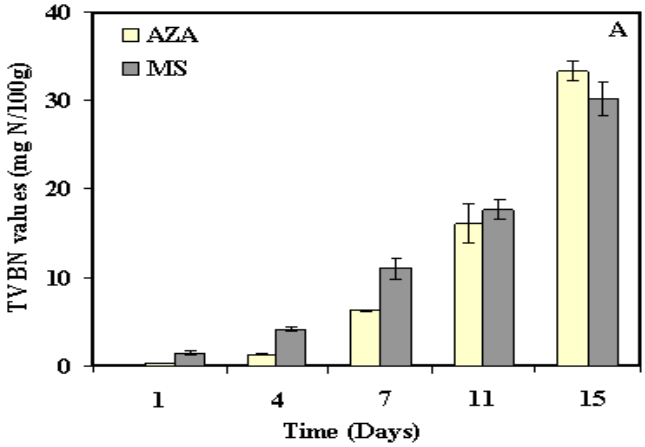

Figure 6: TVBN at 4°C (A) for sausages treated with herbs extract AZA and Sodium metabisulphite.

3.6 Sensory analysis: Sausages treated with the herbs, were not significantly different (P>0.05) in the sensory evaluation ratings. However, AZA and sodium metabisulphite were rated 6.8 and 7.2 respectively, indicating they were "moderately good. All sausages were found to be of moderately good overall acceptability.

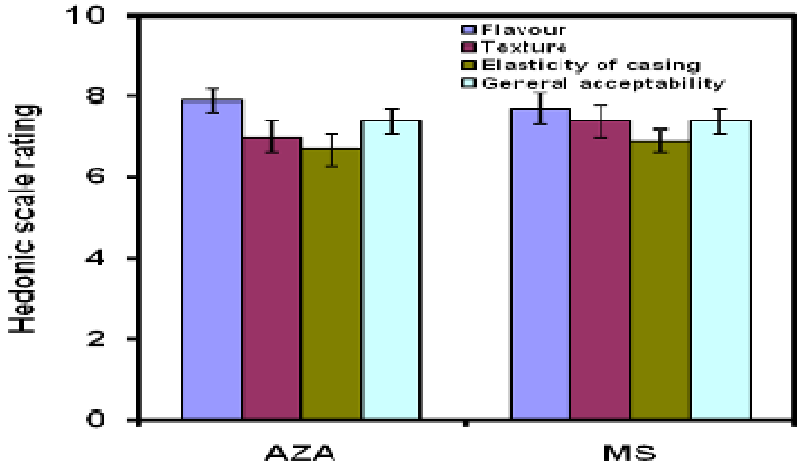

Figure 7: Mean sensory hedonic scores for sausages with herb extracts, sodium metabisulphite (MS), and a control

The panellists were not able to distinguish the taste of AZA from the control meaning that the herb AZA did not impart any unusual taste to the sausages (Fig; 9). This concurred with survey findings where the pastoralists of West Pokot claimed that the herb AZA did not impart any taste to the meat. The extracts of AZA have potential as preservative for use in meat products (Jellinek 1985).

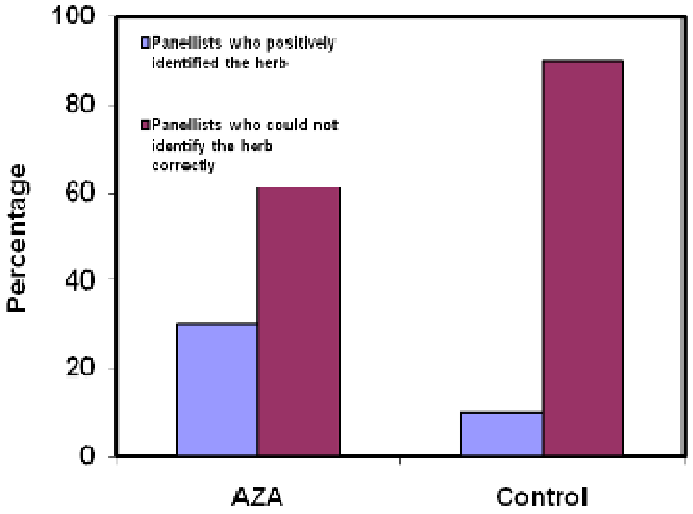

Figure 8: Ability to distinguish sausages treated with the herbs extracts.

4 CONCLUSIONS AND RECOMMENDATIONS

The study established that AZA commonly used to preserve meat is a good antioxidant with moderately good antimicrobial properties and can preserve sausages for at least 10 days at 4°C. The study concluded that at 25°C, sausages could only be stored for one day with or without a preservative. Sodium metabisulphite is a more effective antimicrobial agent than AZA, while AZA is a more effective antioxidant. Sensory evaluation indicated that the herb AZA did not impart its taste on the sausages and therefore did not alter the original taste of the sausages.

4.1 Areas for further research: Further research can be done to identify the active phytochemical compounds responsible for the antimicrobial and antioxidant activity and formulate products for commercialization.

5 ACKNOWLEDGEMENT

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