GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL 16, NO. 3 2010: 281-287
 ISSN 1118-0579
www.globaljournalseries.com; Email: info@globaljournalseries.com

INFLUENCE OF LOCAL SPICES (*Tetrapieura tetraptera and Allium sativum*) ON THE KEEPING QUALITY OF SAUSAGES PREPARED UNDER LABORATORY CONDITIONS

C. U. OKORO AND S. P. ANTAI

(Received 20 September 2006; Revision Accepted 14, June 2010)

ABSTRACT

Two local spices *Tetrapleura tetraptera and Allium sativum* (garlic) were used as preservatives in the preparation of chicken and beef sausages. The effect of these spices on the microbiological and biochemical properties of sausages was monitored. Initial microbial counts of ground meat before treatment with preservatives were 2.55 x 10^5cfu/g and 3.64 x 10^5cfu/g for raw beef (Br) and raw chicken (Cr) respectively. Ground meat was treated with garlic (g), *Tetrapleura tetraptera* (t), sodium nitrite (n) or their combinations (gn, tn) at varying concentrations for the preparation of different sausage samples. Storage was carried on at two different temperatures and results showed that sausages treated with 4% garlic, 4% *Tetrapleura tetraptera* and 0.42% sodium nitrite per 100g meat showed much proliferation of microbes during storage. For these samples microbial counts were 3 x 10^3cfu/g, 1.4 x 10^4cfu/g, 2 x 10^3cfu/g, 1.3 x 10^3cfu/g for Bg_1, Bt_1, Cg_1, and Ct_1 respectively. Microbial genera isolated were mainly *Escherichia coli, Staph. aureus, Lactobacillus bulgaricus*, *Micrococcus spp* and *Rhizopus*. Sausages treated with 10% garlic, 10% *Tetrapleura tetraptera* and 0.42% sodium nitrite per 100g meat were less susceptible to microbial deterioration irrespective of whether the sausages were stored at room temperature or refrigeration temperature. For these samples, post processing counts were 1 x 10^3cfu/g, 1 x 10^3cfu/g, 2.7 x 10^4cfu/g, 1 x 10^3cfu/g, 5 x 10^3cfu/g and 2 x 10^3cfu/g for Bg_2, Bt_2, Cg_2, Cgn, Ct_2 and Ctn respectively. The microbial genera isolated after treatment were *Lactobacillus bulgaricus and Staph epidermidis*. *Escherichia coli, Staph. aureus,* were absent. Btn and Bgn had no microbial growth. All sausage samples showed reduced percent fat and increased ash, protein and carbohydrate content.

KEY WORDS: *Tetrapleura tetraptera, Allium sativum Micrococcus spp, Staph epidermidis, Lactobacillus bulgaricus*

INTRODUCTION

Meat and meat products are rich in nutrients. Some of these nutrients especially the low density lipids (LDL), triglycerides and cholesterol can lead to some nutritional diseases like artheriosclerosis and coronary heart diseases when consumed in large quantities. Silagy and Neil (1994) discovered that garlic (*Allium sativum*) lowers the level of low density lipids and triglycerides which are the major causes of increased blood cholesterol in man. Both fatty acids have been implicated in raised blood pressures. The Food and Agricultural Organization has observed that muscle meat is high in fat content especially the low density lipids. Pork trimmings for instance consist of more than 40% blood pressures. The Food and Agricultural Organization has observed that muscle meat is high in fat content especially the low density lipids. Pork trimmings for instance consist of more than 40% fat. Lean beef trimmings and lean beef cuts are preferred for use in fermented sausage products (Savic, 1985). Raw meat and poultry come from warm-blooded animals which carry hetergenous pathogenic bacteria (Beuchat et al., 2001). Microbial contaminants of meat are derived from the air, environment of the abattoir, the slaughter slabs and knives, the hides and hooves, the gastrointestinal tract of the animal, the wash water and from general hygienic practices (Johanson et al., 1983).

Organisms which have been found in meat according to Patterson and Gibbs (1978) include *Bacillus spp, Coryneforms, Microbacterium thermosphactum* and coagulase negative cocci such as *Staphylococcus epidermidis; Pseudomonas spp* and the Enterbacteriaeceae (Citrobacter) Enterobacter. Izuminoto et al; (1983) reported that micro-organisms found in fresh ground beef included *Pseudomonas spp* and the *Enterbacteriaeceae* and *Lactobacillus spp*. These biochemical and microbiological qualities of meat present a hazardous scenario for meat lovers. Luckily some spices have been found to posses antimicrobial and lipolytic properties which could help to make meat products nutritionally good for all. Al-Delamy and Ali (1970) reported that extracts of various spices, have been found to be excellent antimicrobials and their use in meat curing mixtures are being tried. They found out that filtered onion extracts were bactericidal to *Shigella dysenteriae* and *Staph aureus*. Amy Bigus et al. (2003) reported that garlic acts as a natural antibiotics and inhibits the growth of *Staphylococcus, Streptococcus, Bacillus, Brucella* and *Vibrio species*. Uraih and Nkanga (1983) discovered that cloves (*Eugenie caryophyllus*) reduced *Staphylococcus spp*. counts. This was attributed to its eugenol content. Bullerman (1974) reported that mycelia growth and aflatoxin production by *Aspergillus parasiticus* were inhibited by cinnamon. Garlic as reported by Silagy and Neil (1994) has been implicated in lowering the level of low density lipids (LDL) and triglycerides.

C. U. Okoro, Department Of Microbiology, University Of Calabar, Calabar –Nigeria.P. M. B. 1115 Nigeria
S. P.Antai, Department Of Microbiology, University Of Calabar, Calabar –Nigeria.P. M. B. 1115 Nigeria

Salako et al., (1990) showed that alcoholic and water extracts of *Tetrapleura tetraptera* inhibited the growth of *Staphylococcus aureus*. Essien et al. (1994) in their part stated that nutritionally *Tetrapleura tetraptera* contains varying amounts of nutrients such as proteins, lipids and minerals. The use of these spices in combination with *Lactobacillus bulgaricus* together with the fermentation process were employed to monitor the shelf-life stability and quality of sausages produced. The sausages were stored in the laboratory under two storage conditions: room temperature (29±1^{o}C) and deep freezer temperature (-2±2^{o}C).

MATERIALS AND METHODS

Meat samples from beef and chicken were purchased from the Calabar abattoir and University of Calabar farm respectively, washed with brine solution, minced in a meat mincer and mixed with preservatives at different concentrations of (4% garlic, 4% *Tetrapleura tetraptera,* 10% garlic, 10% *Tetrapleura tetraptera* and 0.42% $NaNO_2$ per 100g meat sample) and then stuffed into sausage casings made of pig intestine. The pig intestine was previously washed with brine solution several times and vinegar to avoid contamination by microbes and worms. The pig intestine was then tied on both ends and the sausages stuffed into it and allowed to fermented at 70^{o}C for 5 minutes, smoked at 80^{o}C for 3 hours and stored on the shelf at between 29±1^{o}C and inside the deep freezer at -2±2^{o}C.

PROCESSING TECHNIQUES

The two sausage samples were prepared using the following processing techniques mincing, mixing, fermentation, steaming and smoking.

Mincing: After cleaning and washing meat samples in brine solution, 300g of meat samples were weighed on a balance and minced in a sterile meat mincer.

Mixing: A 100g weight of the minced meat sample was transferred into a mixer-blender and mixed thoroughly with 10ml of the starter culture 100mls of sterile water along with all ingredients were also mixed together in the blender to form a uniform consistency. The sausage ingredients were sodium nitrite, sodium chloride and glucose.

Addition of Local Spices: Different measures of local spices was added to the sausage mixture. Water extracts of garlic and *Tetrapleura tetraptera* were prepared by pounding in a sterile mortar. A 4g weight of each of the ground spices were mixed with 10mls of sterile water and filtered. The filtrate was then poured into the sausage mixtures. This was repeated using 10g of each of the local spices.

Fermentation: This process was carried out in the oven at 37^{o}C for 2 days.

Steaming: Fermented sausage samples were stacked on a warm steamer at 70-80^{o}C for 15 minutes and allowed to coagulate before smoking.

Smoking: Fermented and steamed sausage samples were transferred to a locally constructed smoke oven and allowed to smoke for 3 hours before storage.

PROXIMATE ANALYSIS

Each sample was aseptically collected with the aid of a sterile spatula and transferred into a sterile grinder and then ground into small pieces for subsequent analysis. Proximate analysis of samples for percent carbohydrate, percent fat, percent ash, percent protein and percent moisture content was carried out using the methods described by Pomeranz and Melon (1982).

ENUMERATION AND ISOLATION OF ORGANISMS

The pour plate method using standard nutrient agar (Difco) was used for the enumeration of total heterotrophic bacteria. Total coliforms were enumerated using Eosin methyl blue (EMB) agar, while pathogenic Staphyllococcus was enumerated using Mannitol Salt Agar (MSA). Microbial number as described by Taylor (1962). Sabourand Dextore Agar (SDA) was used for enumerating fungi. Plates were incubated at 37^{o}C for 24 hours for bacteria or at room temperature for 5 days for fungi.

IDENTIFICATION OF ISOLATES

Identification of enumerated microbes was carried out using morphologic and biochemical characteristics of microbes as described by Kaiser (1999). The microbiological tests were Gram stain, motility, shape, while the biochemical tests included coagulase, indole, catalase, V-P tests and fermentation of sugars.

RESULTS

Results of the analysis for moisture, protein, fat, carbohydrate and ash in the two types of sausage samples is presented in Table 1. Almost all the nutrients recorded increased levels but there was a decrease in percent fat content after processing. Sausages prepared with higher concentration of local spices showed increased ash but lower fat content especially in garlic preserved sausages.

Table 1: Proximate Analysis Of Meat and Sausage Samples

PERCENT NUTRIENT CONTENT	SAMPLES			
	Raw beef	Raw Chicken	Beef Sausage	Chicken Sausage
Protein	18.5	21.3	32.2	45.3
Fat	19.3	12.8	17.1	2.8
Ash	1.4	1.0	6.5	7.2
Moisture	60.9	64.1	20.1	30.8
Carbohydrate	0	0	14.3	13.8

The total heterotrophic bacterial counts were determined before and after processing. Raw beef and raw chicken had counts of 2.55 x 10^5cfu/g and 3.65 x 10^5cfu/g respectively. After treatment with preservatives, there was a slight drop in the counts. Beef treated with 4% garlic or 4% *Tetrapleura tetraptera* or 0.42% sodium nitrite per 100g meat had bacterial counts of 2.06 x 10^5cfu/g and 2.50 x 10^5cfu/g for Bg_1, Bt_1 and Bn respectively. Chicken treated with the same amount of preservatives had 3.12 x 10^5cfu/g for Cg_1, 3.27 x 10^5cfu/g for Ct_1 and 3.00 x 10^5cfu/g for Cn. Figures 1 and 2 show the pre and post processing counts for all treatments. When the concentration of the preservatives was increased to 10% garlic, 10% *Tetrapleura tetraptera* and the combination of local spices with 0.42% nitrite per 100g meat there was a further drop in the microbial counts after processing and during storage. In Figure 2, samples recorded a drop in microbial count after the concentration of preservatives were increased. Btn and Bgn sausage samples had no growth. Bg_2, Cgn and Ctn had 1 x 10^3cfu/g, 2 x 10^3cfu/g and 2 x 10^3cfu/g respectively Ct_2 had 5 x 10^3cfu/g while Cg_2 had the highest number of counts of 2.7 x 10^4cfu/g post processing.

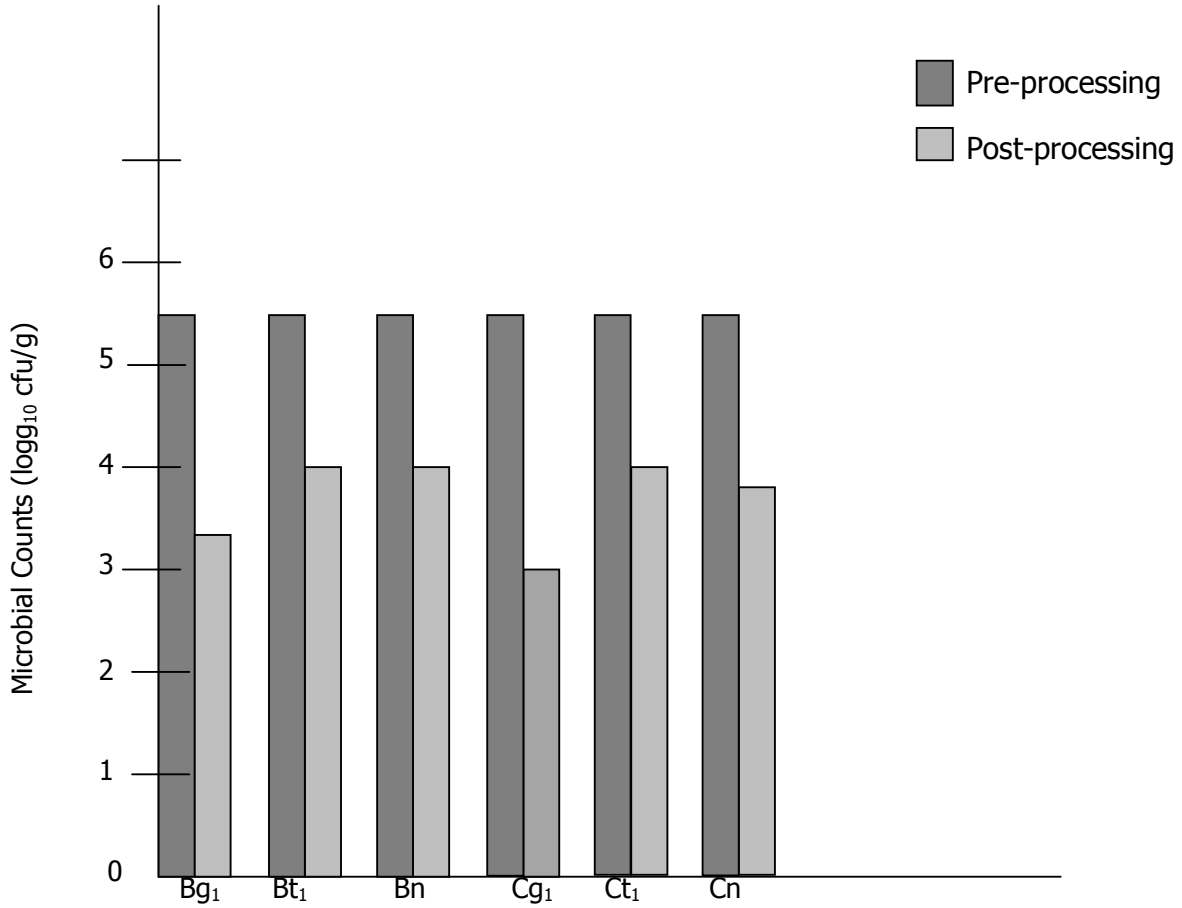
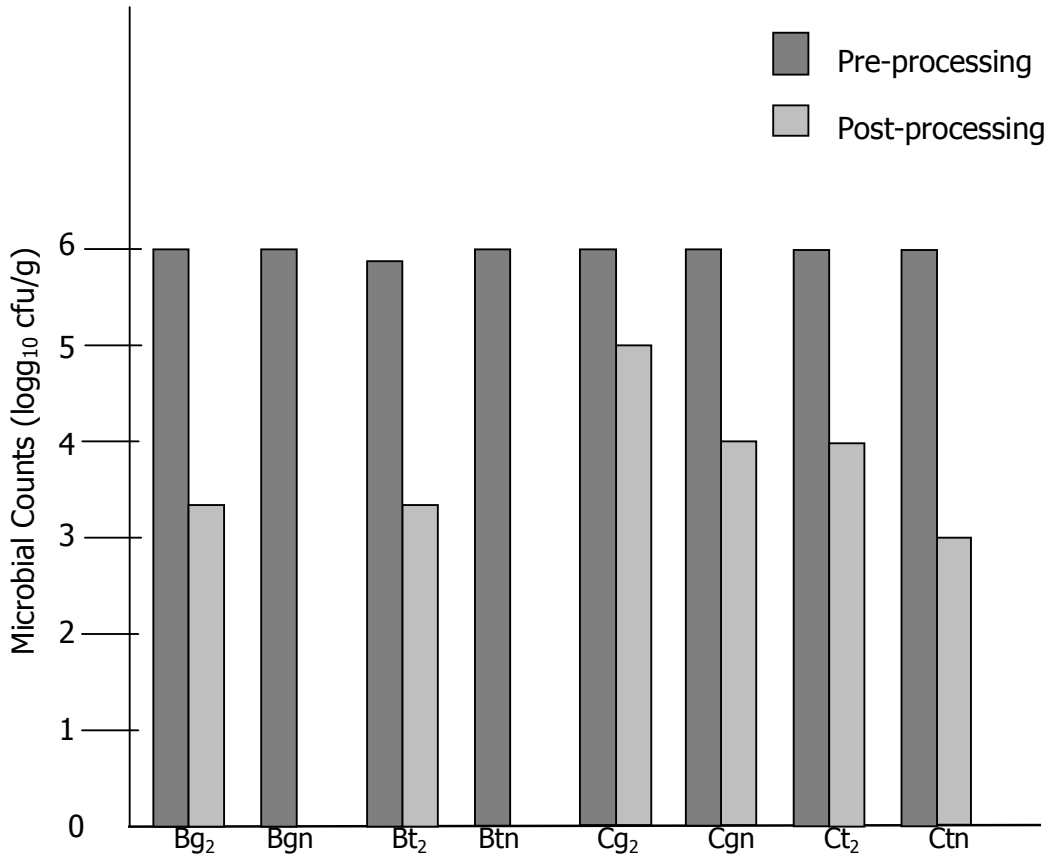


Fig. 1: Effect of treatment on keeping quality of sausages after 1 week shelf storage

Bg_1 = Beef sausage treated with 4% garlic
Bt_1 = Beef sausage treated with 4% *Tetrapleura tetraptera*
Bn = Beef sausage treated with 0.42% sodium nitrite
Cg_1 = Chicken sausage treated with 4% garlic
Ct_1 = Chicken sausage treated with 4% *Tetrapleura tetraptera*
Cn = Chicken sausage with 0.42% sodium nitrite



Sausage Samples

Fig. 2: Effect of higher levels of preservatives on the keeping quality of sausage samples after 1 week shelf storage

Bg_2 = Beef sausage treated with 10% garlic
Bgn = Beef sausage treated with 10% garlic and 0.42% $NaNO_2$
Bt_2 = Beef sausage treated with 10% *Tetrapleura tetraptera*
Btn = Beef sausage treated with 10% *Tetrapleura tetraptera* and 0.42% $NaNO_2$
Cg_2 = Chicken sausage treated with 10% garlic
Cgn = Chicken sausage treated with 10% garlic and 0.42% $NaNO_2$
Ct_2 = Chicken sausage with 10% *Tetrapleura tetraptera*
Ctn = Chicken sausage treated with 10% *Tetrapleura tetraptera* and 0.42% $NaNO_2$

DISCUSSION

The main ingredient for sausage manufacture is meat. The pathogenic species found in meat according to Beuchat et al. (2001), comes from the animal, the environment and some bacterial species introduced during slaughter. The presence of these microbes in meat hastens meat deterioration and decay, but processing and addition of some preservations help in prolonging its shelf life. The use of local spices (garlic and *Tetrapleura tetraptera*) and processing techniques like curing (with NaCl and $NaNO_2$), fermentation and smoking help in prolonging the shelf life of meat and improving its nutritional quality. In Table 1, proximate analysis of sausage samples recorded increased protein, ash and carbohydrate and reduced fat contents. Essien et al. (1994) had reported a high protein and mineral content of *Tetraptera tetrapleura*. Local spices like garlic (*Allium sativum*) and *Tetraptera tetrapleura* are acceptable both in the northern and southern parts of Nigeria as preservatives and food condiments. The concentrations of each spice in both sausage mixes had varying effects on the microbial counts of sausage samples. When sausage samples were treated with lower concentration of garlic (4% per 100g meat) either in beef or chicken (Bg of Cg) we see in Figure 1 that a reduced number of microbes was observed. The percent survival when compared with the pre-processing count of 2.0×10^5cfu/g and 3.12×10^5cfu/g for Bg and Bt is 0.01% and 0.006% respectively. Increased concentration of garlic (10% per 100g meat) in Figure 2 shows almost zero microbial counts of 1×10^3cfu/g and 5×10^3cfu/g for Bg_2 and Cg_2 which could be attributed to contamination. Isolation of organisms for samples treated with garlic showed no presence of *Staph, aureus* and *E.coli* but few colonies of *Staph epidermidis* and *Lactobacillus bulgaricus*, the initial fermenting bacterium. This result confirms the discovery of Amy Bigus et al. (2003) who claimed that garlic acts as a natural antibiotic due to the presence of a chemical component called allicin. Allicin can inhibit the growth of *Staph aureus* as well as *E.coli*. Similarly at lower concentration, sausage samples treated with *Tetrapleura tetraptera* namely Bt and Ct had 7.6% and 3.9% surviving microbes respectively when compared with pre-processing counts. With this we can see that garlic exhibited greater potency in eliminating pathogens in meat even at low concentrations. In Figure 2, when the concentration of *Tetrapleura tetraptera* was increased, Bt_2 and Ct_2 had 0.05% and 1.6% microbial survivors respectively when compared with the pre-processing counts. It then implies that treating meat with higher levels of *Tetrapleura tetraptera* exhibits greater antibiotic effect on the microbes. Salako et al. (1990) reported that alcoholic and water extracts of *Tetrapleura tetraptera* inhibits the growth of *Staphylococcus aureus*. All samples treated with this spice had no *Staph aureus*. Combining either garlic or *Tetrapleura tetraptera* with nitrite in producing sausage samples like Btn, Bgn, Ctn and Cgn at increased concentration of local spices brought about a complete elimination of pathogens in Btn and Bgn and about 2×10^3cfu/g survivors in Ctn and Cgn respectively. These microbes were discovered to be contaminants of *Micrococcus spp* probably from the air.

REFERENCES

Al-Delamy, K. S., and Ali, S. H., 1970. Antibacterial action of vegetable extracts on the growth of pathogenic bacteria. Journal of the sciences of food and agriculture. 21:110-115.

Amy Bigus, Deanna Massengill and Christy Walker., 2003. Garlic ("Allium satium"). http://www.geoities.com/chadix/garlic/html.

Bullerman, L. B., 1974. Inhibition of aflatoxin production by cinnamon. Journal of food science. 39:1163-1165.

Beuchat, L. R. Busta, F. F. and Farbe, J. M. [eds]., 2001. Analysis and evaluation of preventive control measures for the control reduction and elimination of microbial hazards on fresh and fresh-cut produce. Department of Science and technology projects. Institute of food technology. Chicago.

Essien, E. U., Izunwanne, B. C., Aremu, C. Y., Eka, O. U., 1994. Plant food for human. Nutrition 45:47- 51.

Izuminoto, M., Umetani, A. and Miura, H., 1983. Effect of anaerobic packaging on the bacterial count and bacterial flora of fresh meat. Japanese journal of *zootechnical* science. 53(10):672-677.

Johanson, L., Underdal, B., Grosland, K., Whelehan, D. P. and Roberts, T. A., 1983. A survey of the hygiene quality of beef and pork carcasses. *Acta veterinarian Scandinavia.* 24(1):1-13.

Kaiser, G. E., 1999. Microbiology laboratory manual.http://www.cat. cc. md.us/courses /bio/41 /labmanualtoc.html.

Patterson, J. T. and Gibbs, P. A., 1978. Incidence and spoilage of isolates from vacuum-packaged meat of high pH value. *Journal of Applied Bacteriology* 43:25-38.

Pearson, D., 1976. The chemical analysis of foods. 7th Ed. Edinburg, London and New York. P6-16.

Silagy, C. A., and Niel, H. A., 1994. “A meta-analysis of the effect of garlic on blood pressure”. Journal of Hypertension 12(4):463-468.

Salako, O., Akpan, U. E., Ette, E. I. C., Essien, E. E. and Ipeaiyeda, O., 1990 Fitoterapia, 61:169-171.

Savic, I. V., 1985. Small scale sausage production.http://www.fao.org/docrep/003/x6556e/x6556E02.htm.

Taylor, J., 1962. The estimation of bacterial numbers by tenfold dilution series. Journal of Applied Bacteriology 25:54-61.

Uraih, N. and Nkanga, E. J., 1983. Influence of food additives on bacterial population, Nigerian Journal of Microbiology, 3(1):46-56.