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# Comparative Microbial Analyses of Freshly Prepared Stored Beef Sausages of Different Vegetable Oils

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## ABSTRACT:
Freshly prepared beef sausage samples using four different types of vegetable oils; lard, olive oil, Shea butter, groundnut oil and a control sausage prepared without any oil were used in this study. The study was partitioned into five treatments based on the type of oil used and labeled, $T_1$ (Control i.e. without oil), $T_2$ (Lard based), $T_3$ (Olive oil based), $T_4$ (Shea butter based) and $T_5$ (Ground nut oil based). Method of preparation for all sausage samples was same and according to a standard commercial method with all ingredients added equally. The sausage samples were stored at $4^0$C for 20 days. Microbial analyses of samples was done every 5 days beginning from the day of preparation (day 0) up to day 20 following the procedures of APHA and Difco manuals using plate count agar (PCA) and MSA (Mannitol Salt Agar). The analyses procedures were replicated three times and the mean data obtained was subjected to the Duncan Multiple Range Test statistical analyses. The bacteria species isolated and identified in the course of the experiment include coliform bacteria, *E. coli* and *Enterobacter spp*., *Staphyloccocus aureus* and *Staphyloccocus spp.* Results of the study indicated a gradual buildup of microbial populations in the samples from day 0 of the experiment. The results also indicated that microbial count increases as storage period increases reaching its peak at day 20 in all sausage samples. All the oil based samples i.e. $T_2 - T_5$ had lower bacterial counts compared to the control sausage without oil ($T_1$).Significant differences ($P < 0.05$) exists in the total plate count among all the treatment from day 0 to day 20.The results shows that olive oil based sausage ($T_3$) and groundnut based sausage ($T_5$) had lower microbial counts of $2.60 \times 10^4$ and $2.30 \times 10^4$ respectively at day 20 compared with those of No oil ($T_1$), Lard ($T_2$) and Shea butter ($T_4$) based sausages with microbial counts of $8.20 \times 10^4$, $3.80 \times 10^4$, and $5.40 \times 10^4$ respectively. Both Olive oil and Ground nut oil effectively inhibit microbial growth on MSA in comparison with other oils as there was no microbial growth recorded on both oils from day 0 to day 20. In the present circumstance, it seems Olive oil and groundnut oil are better oils for sausage manufacturing as they seem to possess better antioxidant and antimicrobial qualities.

**Keyword:** *Microbial, stored, Beef, Sausages, Comparative.*

## INTRODUCTION:
Sausages are comminuted and seasoned meat product that may be cured, smoked, molded and heat processed. (Judge *et al*, 1990). Sausages are products in which comminuted meats are modified by various processing methods to yield desirable organoleptic and keeping properties. Sausages are one of the oldest forms of meat processing and modern sausage technology has its roots duly embedded in history (FAO, 1992). Sausages are made from chopped or comminuted lean meat and fat mixed with salt, spices and other ingredients, and then filled into a casing made of animal intestine or cellulose. Sausages occur in two forms; raw sausages and heat processed sausages according to methods applied in their preparation. Raw sausages are further subdivided into two categories; fresh sausages and fermented sausages while heat processed sausages are classified into smoked precooked sausages, emulsion type sausages and cooked sausages. Fresh and smoked pre-cooked sausages are mostly cured and non-fermented. Their shelf life is increased by heating due to partial reduction of their moisture content. They are cooked before consumption. Emulsion-type sausage are made from comminuted and well homogenized cured meat, fatty tissues, water and seasonings, usually smoked and slightly cooked. Cooked sausages are ready to serve products made from previously cooked fresh or cured and subjected to final cooking after stuffing with or without additional smoking

The major factors used in determining the quality of fresh sausages are colors, stability, and general appearance, cooking and eating properties (Mohammed, 1996). Sausages are delicious and nutritious due to their high protein contents, fats, vitamins and minerals (Judge *et al*., 1990). Hams and sausages are the most popular processed meat products (Brown, 2004). Meat processing aids in producing varieties and convenient meat products. The need for effective cheap and simple preservative technique cannot be ignored and one of such is intermediate moisture food processing (Omojola, 2008). Even though meat from freshly slaughtered, healthy animals is supposed to have no or very low microbial populations, laboratory evidence suggests that they could be contaminated at the point of consumption (Umoh, 2011).During slaughtering, subsequent meat cutting and initial processing steps, the numbers of microorganisms in meat are steadily increasing (Gunter and Peter, 2007).

The nutritive and calorific values of seeds make them good sources of edible oils and fat diets (Akubugwo, et al., 2008 and Odoemelam, 2005). Vegetable oils are derived from plant sources like soybean, melon, groundnut, corn, palm oil, Shea butter, coconut, castor oil etc. (Siyanbola, et al., 2013). Vegetable oils are composed of triglycerides which are the ester of one molecule of glycerol and three molecules of fatty acid. Fatty acids are primary nutritional components found in edible seed oils. They are liquid substances at room temperature (Parwez, 2011).They are mainly classed as oleic – linoleic acid oils since they contain a relatively high proportion of unsaturated fatty acid, such as the monounsaturated oleic acid and the polyunsaturated linoleic acids (Nkafamiya, et al., 2010, Musa et al., 2012).

Vegetable oils are characterized by a higher ratio of polyunsaturated fatty acids to saturated fatty acid. They contain very high concentration of omega 6 fatty acids and polyunsaturated fats. Nutritionally, they are preferred to animal fat as evidence linking health benefits to the consumption of

vegetable oils continue to grow (Parry et al, 2005). Vegetable oils contain additional flavornoids, tamin, terpenoids, anthraquinone etc. and majority of these phytochemicals are known to have valuable therapeutic activities such as insecticides (Kanbul, et al, 1982), antibacterial and antifungal (Lemos et al, 1990 and antioxidant (Vardar- unlu et al, 2003). Vegetable oils also contain trace elements like Na, K, Ca, Mg, Fe, Cu, Zn and Mn which are essential nutrients for human growth.

The quality attributes of meat and meat products have been noted to deteriorate due to lipid oxidation and microbial growth. Lipid is responsible for reduction in nutritional quality as well as changes in flavor (Agurrezabal et al, 2000). Microbial contamination can precipitate major public health hazards and economic loss in terms of food poisoning and meat spoilage. Therefore application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf – life and preventing economic loss (Yin, 2003). Research also indicated that lipid oxidation and microbial growth in meat products can be controlled or minimized by the use of synthetic or natural food additives (Giayjomaa and Buckley, 1996, Lee et al, 1997, Meilink et al, 2003).The increasing resistance of microorganisms to conventional chemicals and drugs has prompted scientists to search for novel sources of biocides with broad spectrum activities (Abad et al, 2007). Since ancient times, plants and their derivatives such as essential oils (EOs) have been used in folk medicine (Bakkali et al; 2008). These plants are known for their antioxidant effects as well as their antiseptic and medicinal properties and fragrance and are often used in the preservation of foods and as analgesics, sedatives, anti – inflammatory, spanolytics and local anesthetics (Bakkali et al; 2008).Essential oils contain a wide series of secondary metabolites that can inhibit or slow the growth of bacteria, yeasts and moulds (Chorianopoulos et al; 2008). The EOs are present in vegetables such as Shea butter, olive, groundnut, corn, soya etc. Their concentration depends on the component of the oil seed that the oils are extracted from. This paper focuses on the comparative effectiveness of different vegetables oils in reducing oxidative and microbial deterioration of beef sausages during storage.

## METHODOLOGY

The investigation was conducted in the microbiology laboratory of the Department of Biological Sciences, Achievers University, Owo.

**Meat Source:**

Semi – membranous muscle from mature (3 year old) bull was purchased from the abattoir immediately after slaughter, pig intestine and lard were also purchased from the market.

**Sausage Preparation:**

The sausages were prepared according to standard commercial methods using the recipes shown in Tables 1 - 3 below. The sausages consist of minced meat to which table salt, dry spices, green spices and cold water were added. The oils that were used for the sausage production were Lard, Olive oil, Shea butter, and Groundnut oil. Ingredients for all categories of sausages and method of preparation were the same and involved the steps shown in the flow chart below (Fig 1). The products were stored in the chiller for 3 weeks.

**Table 1**: **INGREDIENTS OF SAUSAGE RECIPES**

| Ingredient | Composition % |
|---|---|
| Beef | 65.00 |
| Lard | 20 .00 |
| Binder | 3. 50 |
| Curing salt* | 2. 00 |
| Sugar | 1. 0 |
| Phosphate | 0. 30 |
| Ice water | 4. 00 |
| Dry spices | 2. 00 |
| Green spices | 2. 20 |
| Total | 100 .00 |

* Sodium chloride and sodium nitrate.

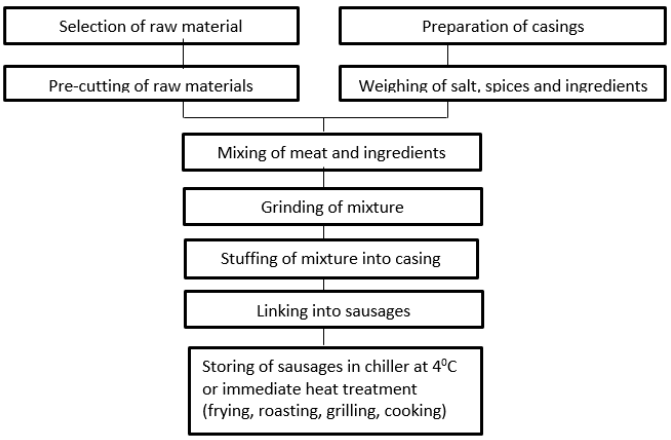
**Table 2**: **COMPOSITION OF DRY SPICESFOR BEEF SAUSAGE**

| Spice | Inclusion level % |
|---|---|
| Black pepper | 20.00 |
| Nutmeg | 7.00 |
| Calabash nutmeg | 3.00 |
| Red pepper | 20.00 |
| Monosodium glutamate | 15.00 |
| Thyme | 20.00 |
| Curry powder | 10.00 |
| Total | 100.00 |

**Table 3**: **COMPOSITION OFGREEN SPICES FOR BEEF SAUSAGES**

| Spices | Inclusion level % |
|---|---|
| Onion (Allium cepa) | 60.00 |
| Ginger (Zingiber officinale) | 20.00 |
| Garlic (Allium sativum) | 20.00 |
| Total | 100.00 |

FIG 1: **FLOW CHART FOR SAUSAGE PRODUCTION**




**Microbial Evaluation:**

The microbiological analyses of sausage samples performed include determination of aerobic bacteria, *Staphyloccocus* and morphological characteristics of the isolates. The procedures of APHA (1992) and Difco Manual (1984) were followed for the determination of total bacterial count using plate count agar (Olutiola et al, 1991). Microbial analyses of the sausages were done every five days beginning from the day of production for the period of 3 weeks to determine oxidative deterioration and microbial load of the sausage samples in three (3) replications.

**Culture Media Preparation:**

The PCA and Mannitol Salt Agar (MSA) used in this experiment was prepared according to manufacturer's specifications and then autoclaved at $121^0$C for 15 minutes.

**Serial Dilution Technique:**

1gm of each sausage sample was macerated in a mortar containing 9mls of sterile distilled water to make the first dilution ($10^{-1}$). Then 1ml from the first dilution ($10^{-1}$) was transferred to a second tube containing 9mls of sterile distilled water ($10^{-2}$ dilution). The process was repeated up to the $6^{th}$ tube,

which gave $10^{-6}$ dilution according to method of Olutiola *et al* (1991).

**Total Plate Count:**

Plate Count Agar (PCA) was used for this analyses. The inoculation was done using pour plate method. Samples of sausage were introduced to the Petri dish and 15mls agar was poured into the plate. Agar and samples were inoculated for 18 to 24 hrs. at 370C. The microbial counts were reported as colony forming units (Cfu)/g sausage sample.

The quantitative enumeration of the organisms as colony forming units per gram was calculated using the formula below:

$$\frac{\sum C \quad X \quad \text{Dilution factor}}{\text{Inoculums size}}$$

**Where**

$\sum C$ = number of colonies.

Dilution Factor = reciprocal of total dilution.

**Selective Plate Count:**

This was done using selective bacterial culture media which contain chemical additives that suppress the growth of all bacteria except the group of microorganisms that was detected and used as indicator bacteria. Colonies were counted to assess the degree of contamination. Enumeration of *Staphyloccocus aureus*, *Staphyloccocus spp.* were done by first sub- culturing colonies on Mannitol Salt Agar (MSA) to obtain pure cultures. Morphological characteristics, gram staining, and biochemical tests of the pure culture were used to confirm the species.

**Morphological Characteristics:**

This was done by direct observation of microorganisms for their shape, opacity, elevation, surface, edge and consistency.

**Gram Staining:**

A heat fixed slide from an 18 – 24 hrs. old culture was prepared. The culture was stained with crystal violet solution for 1 – 2 minutes .It was then rinsed with Gram's iodine solution and the slides were washed with 95% alcohol until no more violet ran from the slide. The slides were washed with water, blot dried and examined under the microscope.

**Biochemical Tests:**

The biochemical tests performed include fermentation of sugar, methyl red test, citrate test, catalase test, oxidase test, coagulase test, indole test and mobility test to identify organisms up to species level

**Statistical Analyses:**

Data generated from the experiment were analyzed using SAS (2000) with means separated with Duncan Multiple Range Test.

## RESULTS

The results of the bacterial status of freshly prepared and stored sausage samples prepared with different vegetable oils are presented in Table 4 below:

**Table 4: Comparative microbial status of freshly prepared and stored sausages produced with different vegetable oils**.

| | | TYPES OF OILS USED FOR SAUSAGES PREPARATION | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | Length of storage (Days) | Control (No oil) | Lard Based Sausage | Olive oil Based Sausage | Shea-butter Based Sausage | Groundnut Based Sausage | |
| | | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** | **SEM** |
| PCA | 0 | $1.70^{ek}$ | $0.00^{ei}$ | $1.60^{dk}$ | $3.00^{cj}$ | $3.50^{bi}$ | *0.23* |
| | 5 | $5.00^{di}$ | $3.20^{bl}$ | $4.00^{ak}$ | $3.80^{bk}$ | $4.50^{aj}$ | *0.22* |
| | 10 | $7.10^{cl}$ | $1.00^{dk}$ | $1.50^{dj}$ | $1.50^{dj}$ | $1.40^{ej}$ | *0.12* |
| | 15 | 7.60ai | $2.40^{ck}$ | $2.10^{cl}$ | $1.20^{em}$ | $2.90^{cj}$ | *0.15* |
| | 20 | $8.20^{bi}$ | $3.80^{ak}$ | $2.60^{bl}$ | $5.40^{aj}$ | $2.30^{dm}$ | *0.20* |
| | SEM | *0.23* | *0.34* | *0.22* | *0.13* | *0.16* | |
| MSA | 0 | $1.20^{ai}$ | $0.00^{ci}$ | $0.00^{aj}$ | $0.00^{cj}$ | $0.00^{aj}$ | *0.11* |
| | 5 | $0.00^{ck}$ | $0.20^{bj}$ | $0.00^{ak}$ | $1.00^{ai}$ | $0.00^{ak}$ | *0.09* |
| | 10 | $0.00^{cj}$ | $0.00^{cj}$ | $0.00^{aj}$ | $0.30^{bi}$ | $0.00^{aj}$ | *0.10* |
| | 15 | $1.00^{bj}$ | $1.30^{ai}$ | $0.30^{ak}$ | $0.00^{ck}$ | $0.00^{ak}$ | *0.90* |
| | 20 | $0.00^{cl}$ | $0.00^{ci}$ | $0.00^{ai}$ | $0.00^{ci}$ | $0.00^{ai}$ | *0.12* |
| | SEM | *0.11* | *0.12* | *0.11* | *0.13* | *0.11* | |

"ijk" along the column with same superscript are not significantly different ($p>0.05$).

"abc" along the row with the same superscript are not significantly different ($p>0.05$).

The results shows that significant differences ($P< 0.05$) exists in the total plate count amongst all the treatments even at day 0 i.e. when the sausages were still fresh on both PCA and MSA. Groundnut oil based sausage had the highest value of 3.50 while lard based had no trace of bacteria with a value of 0. 00. (Table 4). Microbial counts increases as storage period increases for all treatments and got to its peak at day 20. At day 20 sausage sample without oil ($T_1$) had the highest microbial count compared with oil based sausages. This indicates that with or without oil, sausages deterioration would occur during storage in consonance with the assertions of Tappel, 1952; Green, 1969; Kesinkel et al, 1964, Younathan, et al, 1959 that fresh meat and many processed meat products sausages inclusive are all susceptible to lipid oxidation. Lipid oxidation is a major problem in many sectors of the food industry.

Lipid oxidation is the major causes of deterioration in the quality of meat and its products. It is responsible for reduction in nutritional quality as well as changes in flavor (Agurrezabal et al, 2000). It has also been observed that refrigerated and frozen fresh meats are also susceptible to lipid oxidation, the results of this study is a confirmation of this assertion as indicated by the presence of microbes in sausage samples from day 0. Control of lipid oxidation in meat and meat products and meat quality preservation has become increasingly important. The results of this study shows that olive oil ($T_3$) and groundnut oil ($T_5$) had the lowest microbial count of 2.60 $x10^4$ and 2.30 $x10^4$ respectively even after 20 days of storage.(Table 4). Both vegetable oils also effectively inhibit microbial growth on MSA as there was no microbial growth recorded in both from day 0 to day 20. It could therefore be concluded that olive oil and ground nut oil possesses antimicrobial substances that effectively prevented deterioration of the beef sausages and by implication reduction of lipid oxidation by acting as antioxidants.

This could be due to the presence of phenolic compounds in these oils and this is in line with Lemos et al, 1990, and Varda – unlu et al, 2003 who both opinionated on the antibacterial and antioxidant nature of vegetable oils. Conclusively therefore the results of this experiment points to the fact that olive and

groundnut oils are the better oils of consideration in beef sausage production. Over the entire period of storage i.e. chilling conditions, olive and groundnut oils inhibit the growth of microbes which is an indication that both oils contain phenolic compounds which act as antioxidant and antimicrobial substances.

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