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Archives of Applied Science Research, 2013, 5 (3):50-56
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Microflora analysis of sellected meat and meat products from Calabar, Cross River State-Nigeria

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ABSTRACT

Meat has long been known for its nutritive composition which could explain why it is being consumed by many people worldwide. Various biochemical changes and micro-organisms are associated with meat, during the process of slaughter, processing and preservation. This work evaluated the micro-flora of selected meat and meat products. Selected meat and ready to eat meat products in calabar, cross river state- Nigeria were collected randomly and analyzed microbiologically, and isolates were identified as Staphylococcus aureus (21.43%), Escherichia coli (14.20%), Streptococcus Spp (14.29%), Salmonella Spp (14.20%), Bacillus Spp (21.43%), Pseudomonas Spp (7.14%) and Proteus Spp (7.14%). The most frequently isolated organisms were Staphylococcus Spp, Escherichia coli and Basillus Spp. The total viable count for bacterial counts ranged from1.4X10^5 to 2.3X10^{5}cfu/g, whereas total coliform counts ranged from 1.0X10^5 to 3.5X10^{5}cfu/g. The results showed that the meat products were contaminated at various stages of preparation. This calls for proper handling and hygiene at every stage of meat preparation.

Key words: microbial analysis, micro flora, meat, meat products.

INTRODUCTION

Meat is defined as “the part of the muscle of any cattle, sheep, swine, or goats which is skeletal or which is found in the tongue, diaphragm, heart, or oesophagus, with or without the accompanying and overlying fat, and portions of bone (in bone-in product such as T-bone or porterhouse steak), skin, sinew, nerve, and blood vessels which normally accompany the muscle tissue and that are not separated from it in the process of dressing” [1]. It is animal tissues that are suitable for use as food and it includes processed foods prepared from those tissues [2]. Meat and meat products are very perishable. Deterioration begins soon after exsanguinations, resulting in microbial, chemical and physical changes. The initial microbial load plays a role in the determination of the food product’s shelf-life. Three major micro-organisms found in meat are fungi, mold, and bacteria, being a major source of contamination [3].

The basis of a good diet - one adequate for growth, development and maintenance of health - is variety; a variety of foods can supply enough of the complete range of nutrient [4]. Much of the malnutrition seen in the world is a result of relying too heavily on a single staple food. Improvements in the diet depend on a knowledgeable selection of foods that complement one another in the nutrients that they supply. It is, however, difficult in many regions to

obtain such variety. Meat can complement most diets, especially those dependent on a limited selection of plant foods [5].

Meat and meat products are concentrated sources of high quality protein and their amino acid composition usually compensates for shortcomings in the staple food. They supply easily absorbed iron and assist the absorption of iron from other foods as well as zinc, and are rich sources of some of the vitamins in the B group [6,7]. By providing such nutrients, meat consumption can alleviate common nutritional deficiencies. The appropriate utilization or expansion of existing sources of meat calls for coherent development of a complex system of production, processing and marketing, including aspects of finance and expertise for construction and operation of meat plants, and means of storage, meat preservation, transport and marketing [8, 9].

In many developing countries, especially Nigeria, meat is widely consumed as source of protein; it is either eaten cooked or processed into other forms to avoid associated spoilage [10, 11]. Meat is defined as 'the edible part of the skeletal muscle of an animal that was healthy at the time of slaughter [12]. Chemically, meat is composed of four major components including water, protein, lipid, carbohydrate and many other minor components such as vitamins, enzymes, pigments and flavour compounds [113]. The relative proportions of all these constituents give meat its particular structure, texture, flavour, colour and nutritive value. However, because of its unique biological and chemical nature, meat undergoes progressive deterioration from the time of slaughter until consumption [12]. Meat is a nutritious, protein-rich food which is highly perishable and has a short shelf-life unless preservation methods are used. Shelf life and maintenance of the meat quality are influenced by a number of interrelated factors including holding temperature, which can result in detrimental changes in the quality attributes of meat [10].

Meat composition and nutritive value

Broadly, the composition of meat, after *rigor mortis* but before post-mortem degradative changes, can be approximated to 75% water, 19% protein, 3.5% soluble, non-protein, substances and 2.5% fat [8]. The proteins in muscle can be broadly divided into those which are soluble in water or dilute salt solutions (the sarcoplasmic proteins), those which are soluble in concentrated salt solutions (the myofibrillar proteins) and those which are insoluble in the latter, at least at low temperature - the proteins of connective tissue and other formed structures [8]. The sarcoplasmic proteins are a mixture of several hundred molecular species.

Several of the sarcoplasmic proteins are enzymes of the glycolytic pathway and may be present in more than one form (isozymes). Proteins of beef consist of essential amino acids such as leucine, isoleucine, lysine, methionine, cystine, phenylalanine, threonine, tryptophan, valine, arginine and histidine; of these the last two are considered essential for infants. Amino acids are important for maintenance and repair of body tissues in human [8]. Meat is a very good source of various micronutrients: low-fat pork contains 1.8 mg iron, 2.6 mg zinc; and pigs' liver contains 360 mg magnesium, 20 mg iron and 60 µg selenium per 100 g. A daily intake of 100 g of meat and liver can supply up to 50% of the recommended daily allowance for iron, zinc, selenium, vitamins B1, B2, B6, B12 and 100% of vitamin A [13]. The importance of meat as an essential source of some

Micro-nutrients is due to the fact that it is either their only source, or they have a higher bioavailability. Vitamins A and B12 occur exclusively in meat and can hardly be compensated for by plant-derived provitamins [14]. Iron has a higher bioavailability from meat than from plants (heme iron), as has folic acid which is nearly 10-fold more, especially from liver or eggs, compared to vegetables [14].

Muscles of healthy animals are regarded as sterile, but the slaughtering and butchering process of animals provides bacteria with an opportunity to colonize meat surfaces. Contamination of meat is a continuing possibility from the moment of bleeding until consumption. In the abattoir itself there are many potential sources of contamination of meat by micro-organisms. These include the animal hide and hair, soil adhering thereto, the contents of the gastrointestinal tract (if inadvertently released during dressing operations), airborne contamination, aqueous sources (the water used for washing the carcass, or for cleaning the floors and equipment), the instruments used in dressing (knives, saws, cleavers and hooks), various vessels and receptacles, and the personnel [15, 16]. Aerosols produced during de-hiding, evisceration, and carcass splitting are also important sources of contamination (Mead, 2004). Air circulated from heavily contaminated refrigeration coils in meat and poultry processing plants is also a major source [17]. The initial microbial load of a carcass surface is determined by the hygiene of the abattoir as well as handling practices [18]. Many food-borne diseases are associated with consumption of meat. The pathogens of concern in fresh and frozen meat and meat products include *Salmonella* spp., *Escherichia coli* and other enterohaemorrhagic *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Campylobacter* spp., *Clostridium perfringens* and the potential for *Cl. botulinum* in cured hams and sausages [20]. The most frequent outbreaks associated with consumption of contaminated meat are caused by *Salmonella* spp., *L. monocytogenes*, and *Y.*

enterocolitica [5, 16]. Some diseases could be associated with consumption of meat depending on the processing techniques and level of hygiene practices adopted.

Suya is prepared basically from boneless meat of animals [21]. Muscles meat of almost any kind can be dried to increase its keeping quality. When food materials are dried or roasted, there is loss of moisture. This reduces the water activity (aw) of the food thereby preventing some bacteria from forming spoilage association. In suya preparation, use of lean meat is necessary since fat becomes rancid during the drying process. Suya preparation process in Nigeria lacks hygienic control and the risk of foodborne infections is very high. Some researchers elsewhere had noticed sporadic cases of gastroenteritis and symptoms of infection after consumption of suya which indicated that the product indeed constitute a food safety risk [22, 23].

Kilishi is an intermediate moisture meat that has a suitable concentration of dissolved solids that binds the moisture in it sufficiently to inhibit the growth of spoilage organism, thus it is a ready-to-eat convenience meat product possessing excellent shelf stability at room temperature, making handling and marketing of the product convenient for consumers and retailers alike [24, 22]. It is a traditional, sun dried Nigerian and Saharan African meat product processed using lean beef in combination with plant ingredients. It contains about 46% meat and 54% non-meat ingredients. A finished product contains about 50% protein, 7.5% moisture, 18% lipid and 9.8% fibre/ash respectively [26].

MATERIALS AND METHODS

Sample collection

Ready to-eat Suya, Kilishi, fresh goat meat, beef and chicken were randomly obtained from Watt market in Calabar, Cross River state-Nigeria. The samples were immediately wrapped in sterile aluminium foil to prevent contamination and transported to the laboratory for microbial analysis.

Sample preparation

The selected meat pieces from each sample were removed from the sterile aluminium foils and mashed in a sterile laboratory type mortar and pestle. One gram (1g) of the mashed meat samples was then weighed and aseptically introduced into 9mL of sterile distilled water, properly shaken before a five (5) steps ten (10) fold dilution was performed.

Microbiological analysis

For Total Heterotrophic counts, a five (5) fold serial dilution was done for each of the selected meat products. One (1mL) each was pipette into plated nutrient agar, MacConkey agar and Sabouraud dextrose agar using the spread plate method. Incubation was at 37^0C for 24-48 hours for nutrient agar and MacConkey agar and 25^0C, 3-5 days for Sabouraud dextrose agar. Developed colonies were counted to obtain total heterotrophic counts; Coliform counts and fungal counts. Isolated colonies were further sub-cultured to obtain pure cultures which were subsequently identified using standard methods as reported by Bichanan, (1974).

Gram reaction to differentiate gram-positive from gram-negative organisms, Staphylococcus aureus and Escherichia coli, as control organisms was used.

The motility test for identification of motile bacteria was carried out using the method of [28], catalase test, oxidas test, indole test, methyl red test and citrate test were carried out using the methods of [29] and [30], while the coagulation test and voges proskauer test were carried out using the methods of [28].

Statistical analyses

The data generated were subjected to statistical analyses using SPSS 16.0 for windows. Means were separated by Duncan's Multiple range tests [31].

RESULTS

The results of microbial analysis of selected meat and meat products from Calabar, Cross River State-Nigeria is as presented in the tables.

Table 1: Microbial counts (105 cfu / g) of selected meat and meat products from Calabar, Cross River state-Nigeria

Samples	Nutrient agar (10^5cfu/g)	Sabouraud agar (10^5cfu/g)
Suya meat	1.4^{a}	1.0^{a}
Kilishi meat	2.3^{b}	1.8^{b}
Goat meat	2.9^{c}	2.0^{b}
Beef meat	3.5^{c}	2.5^{bc}
Chicken meat	2.4^{b}	1.5^{ab}

Mean followed by different superscripts within columns are different ($P \leq 0.05$)

Table 2: cultural characterization of bacterial isolates in selected meat samples from Calabar, Cross River state-Nigeria

Samples	Isolates	Colour	Shape	Elevation	consistency
Suya meat	1	Yellow	Round	Raised	Moist
	2	Cream	Circular	Convex	Moist smooth
Kilishi	1	Cream	Circular	Convex	Moist slimy
	2	Cray	Irregular	Flat	Moist
Goat meat	1	Palegreen	Irregular	Flat	Dry
	2	Yellow	Round	Raised	Moist
Beef meat	1	Cream	Round	Raised	Slimy
	2	White	Irregular	Flat	Warming
Chicken meat	1	Cream	Circular	Convex	Moist
	2	Yellow	Round	Raised	Moist

Table 3: Frequency of occurrence of bacterial isolates in selected meat samples from Calabar, Cross River state-Nigeria

Bacteria isolate	Frequency	Percentage
Staphylococcus spp	18	21.43%
Escherichia coli	12	14.29%
Salmonella spp	12	14.29%
Bacillus	18	21.43%
Pseudomonas	6	7.14%
Streptococcus	12	14.29%
Proteus	6	7.14%
Total	**84**	**100**

Table 4: Characterization and identification of isolates from selected meat and meat products from Calabar,. Cross River State-Nigeria.

Micro-organisms	Gram stain	Motility	Indole	Methyl red test	Voges proskauer	Citrate	Catalase	Oxidase	Coagulase	Lactose	Sucrose	Glucose	Manitol	Probable organisms
Cocci cluster	+	-	-	-	+	-	+	-	+	+	+	+	-	*Staphylococcus spp*
Rod	-	+	+	+	-	-	+	-	-	+	+	+	+	*E. coli*
Capsulated rod	-	+	-	+	-	+	+	-	-	-	-	+	+	*Salmonella spp*
Bacillus rod	+	+	-	-	+	+	+	+	-	-	+	+	-	*Bacillus spp*
Carved rod	-	+	+	-	+	+	+	+	-	-	-	+	+	*Pseudomonas aeroginosa*
Cocci in cluster	+	-	-	-	+	-	+	-	+	+	+	+	-	*Staphylococcus aureus*
Bacilli rod	+	+	+	-	+	+	+	+	-	-	+	+	-	*Bacilli spp*
Cocci in chain	+	-	+	-	-	-	-	-	-	+	-	+	+	*Streptococcus spp*
Bacilli rod	+	+	+	-	+	+	+	+	-	-	+	+	-	*Bacilli spp*
Short rod	-	+	+	+	-	-	+	-	-	+	+	+	+	*E. coli*
Pleomorphic rod	+	+	-	+	-	+	+	-	-	-	+	+	-	*Proteus spp*
Cocci in chain	+	-	-	-	-	-	-	-	-	+	-	+	+	*Streptococcus spp*
Capsulated rod	-	+	-	+	-	+	+	-	-	-	-	+	+	*Salmonella spp*
Cocci in cluster	+	-	-	-	+	-	+	-	+	+	+	+	-	*Staphylococcus aureus*

\- *negative result*
\+ *positive result*

DISCUSSION

Meat basically contains all the nutrients necessary for microbial growth and metabolism, making it susceptible to microbial contamination. In view of this, micro-flora of selected meat and meat products must be ascertain to ensure safety from infections after consumption of such products and to promote quality control.

Laboratory analysis carried out on some selected meat and meat products, randomly collected from Watt market, Calabar, Cross River state-Nigeria, indicated contamination of meat samples with various bacteria species, including *Staphylococcus aureus* and some enteric bacteria. [32] also affirmed that meat contain certain amount of salt, and by so permit the growth of *Staphylococcus aureus* whereas, the presence of some members of the enteric bacteriacea family is due to contamination from intestine of slaughtered animal.

Seven (7) organisms were isolated from the selected meat and meat products samples. In view of the unhygienic condition of meat handling in Nigeria, the organisms isolated would always be suspected in connection with meat contamination and spoilage [22, 23]. The organisms include *Staphylococcus spp, Streptococcus spp, Escherichia coli, Salmonella spp, Bacillus spp, Pseudomonas* and *Proteus*.

The presence of *Staphylococcus* spp affirmed the cross contamination through processing [32], since it is a normal flora of the skin. Raw meat is carried on the body by butcher in Nigeria due to lack of hygiene and adequate education [34]. Confirmed coliform often results from the water used for washing the meat, which may have been contaminated. Also presence of *E. coli* probably may have arising from the use of non-portable water during washing of raw meat, as confirmed by [33]. The meat also showed the presence of *pseudomonas aeroginosa*, which usually occur around soil, vegetation and even surfaces [34].

The main critical point of contamination of meat and meat products are roasting, holding and reheating. Control of contamination can be achieved if aseptic techniques of meat preparation processes are observed.

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