

RESEARCH ARTICLE

Preliminary Bacteriological Evaluation of Smoked Rabbit Meat Sold on Lagos-Benin Expressway, Nigeria

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Abstract

Rabbit meat is a lean meat rich in proteins of a high biological value and it is characterized by high levels of essential amino acids. Presence of potential pathogens could, however, pose serious public health risks. This study was carried out to investigate the bacteriological quality of smoked rabbit meat sold on Lagos-Benin Expressway, Nigeria. A ten-fold dilution of rabbit meat samples was prepared up to 10^{-6} factor. Total viable bacterial count (TVBC), Total enterobacteriaceae count (TEC), Total coliform count (TCC), Total *Staphylococcus aureus* count (TSAC) and *Escherichia coli* were determined on plate count agar, violet red bile glucose agar, MacConkey agar, Mannitol salt agar and Eosin methylene blue agar, respectively. Enterococci, Micrococci and *Salmonella* spp were counted on Slanetz Bartley medium, Baird Parker agar and *Salmonella-Shigella* agar, respectively. Biochemical tests were performed for further identification of isolates according to standard methods. Results revealed that TVBC, TEC, TCC and TSAC ranged from 1.48×10^5 to 3.20×10^5 cfu/g, 1.05×10^3 to 1.36×10^3 cfu/g, 1.10×10^3 to 1.44×10^3 cfu/g and 1.14×10^2 to 2.36×10^2 cfu/g, respectively. *Staphylococcus aureus* had the highest percentage frequency of 30.3% while *Salmonella* spp, *Pseudomonas aeruginosa* and *Proteus* spp had the lowest with 6.1% each. Data obtained from certain locations showed significant differences from others ($P < 0.05$). It was revealed from this study that rabbit meats sold on Lagos-Benin Expressway, Nigeria are of satisfactory quality from bacteriological point of view.

Keywords: Bacteria; Food Safety; Public Health; Meat Quality; Rabbit Meat

Introduction

In recent years, much attention has been paid to the influence of diet on human health and well-being. The primary role of diet is to provide sufficient nutrients to meet the nutritional requirements of an individual. There is now increasing scientific evidence to support the hypothesis that some foods and food components have beneficial physiological and psychological effects over and above the provision of the basic nutrients [1]. Many traditional foods contain components with potential health benefits. In addition to these foods, new foods are being developed to enhance or incorporate these beneficial components due to their health benefits or desirable physiological effects. Consumers' interests in the relationship between diet and health have increased the demand for information about functional foods. However, no universally accepted definition for functional foods exists.

The nutritional value of rabbit meat has been reviewed by several authors, showing that rabbit meat has a high nutritional value compared with other meats [2-5]. The main components of meat, excluding water, are proteins and lipids. Rabbit meat is a lean meat rich in proteins of a high biological value and it is characterized by high levels of essential amino acids [3]. Furthermore, meat is an important source of highly available micronutrients, such as vitamins and minerals. Also, rabbit meat does not contain uric acid and has a low content of purines [6]. The information available on chemical composition of rabbit meat is extremely variable, especially regarding fat content, depending on the part of the carcass studied and also on the different productive factors, especially feeding factors having a strong influence on the chemical composition of rabbit meat, in particular, on its lipid composition [7,8].

Rabbit meat is characterized by its lower energetic value compared with red meats due to its low fat content. Fat content varies widely depending on the carcass portion from 0.6 to 14.4% (fat from edible meat with intramuscular and intermuscular fat content) with an average value of 6.8% with the loin being the leanest part of the carcass (1.2% of lipids) [3,5]. Fatty acid composition of rabbit meat is characterized by high polyunsaturated fatty acid content. The fatty acid composition of rabbit meat and its possible

modification through diet will be discussed later on. The amount of cholesterol in rabbit meat is about 59 mg/100 g of muscle, lower values than those presented in meat from other species (61 mg in pork, 70 mg in beef, 81mg in chicken) [2,3].

Rabbit meat production and processing involve a series of interrelated steps designed to convert rabbits into ready-to-cook whole carcasses, cut-up carcass parts, or various forms of deboned meat products. This makes the product prone to microbial contamination. The safety of meat has been at the forefront of societal concerns in recent years. Numerous crises including Bovine Spongiform Encephalitis (BSE) in bovine, high dioxin levels in chicken, the danger of increased spread of other infectious disease (e.g. Foot and Mouth disease, Avian influenza, etc.), as well as pathogens, such as *Salmonella*, *Listeria monocytogenes*, *Campylobacter* and *Escherichia coli* 0157:H7, have reportedly contaminated the European livestock and meat chains. Major meat safety issues and related challenges include microbial pathogens, food additives and chemical residues, and animal identification and traceability [9]. Safety and shelf life of rabbit meat are limited by microbial growth. Dominant contaminants on carcasses and packed rabbit meat are *Pseudomonas*, lactic acid bacteria, yeasts and *Brochothrix thermosphacta*, with total bacteria counts between 4.01-4.96 log cfu/g [10]. It is established that microbial levels of 6-7 log cfu/g are critical for the spoilage of meat [11].

Rodríguez-Calleja, *et al.* [12] studied the shelf life of rabbit carcasses, overwrapped with oxygen-permeable film and stored at 3 °C, over 8 days. Shelf life according to both appearance and odor was estimated at 6-8 days reaching the aerobic plate counts values of 8 log cfu/g. In fact, after 5 days of storage, most of the carcasses already showed some softening and the counts of these bacteria were about 7 log cfu/g. Other author estimated shelf life of rabbit carcasses in 3 days at 4 °C, [13]. These differences could be explained by differences in initial microbial counts, since a high initial contamination of meat reduces product shelf life [14]. In addition, it is possible to increase the shelf life of rabbit meat using modified atmospheres or irradiation [15-17].

Microbial ecology of rabbit meat could also be affected by different feeding programs; some components of the feed could play a specific role on the growth rate of some microbial groups. Vannini, *et al.* showed that a dietary supplementation of whole linseeds limited the growth rate of several microbial groups (except psychrotrophic bacteria) with a consequent increase in meat shelf life. In addition, dehydrated alfalfa meal at high percentages in the diet seems to also have an inhibiting effect on microbial growth in rabbit meat products [18,19].

The slaughtering process may cause extensive contamination of muscle tissue with a vast range of micro-organisms. Some of these micro-organisms come from the animal intestinal tract and others from the environment in contact with the animals before or during slaughter. López, *et al.* have studied the evolution of the most important contaminant and pathogen biota on carcasses during the slaughter process of rabbits [20]. These authors found that there was an increase of microorganism counts during evisceration process, especially enteric micro-organisms, so an improvement of this process is required in order to reduce the final contamination of carcasses. After carcass chilling, microbial counts were reduced to a great extent. Nevertheless, final counts of total aerobic microorganisms and yeasts and moulds were still high. *Listeria monocytogenes*, *Salmonella* spp. and *Campylobacter* spp. were not found in all steps of the slaughter process. *Staphylococcus aureus* was present during evisceration. However, after chilling this microorganism was not present. This study was carried out to investigate the bacteriological quality of smoked rabbit meat sold on Lagos-Benin Expressway, Nigeria.

Materials and Methods

Sample Collection

Smoked rabbit meat samples were purchased from ten different vendors on Lagos-Benin expressway. These were collected into separate sterile plastic bags, put in ice-pack and transported to the laboratory immediately for bacteriological analyses. Samples that could not be transported immediately were stored at 4 °C, for no longer than 24 hours [21].

Bacteriological Analysis

A ten-gram sample was weighed, introduced into a mixer with a sterile spatula under aseptic conditions, and then homogenized by adding 90 ml peptone (water 0.1%). One ml portion of each homogenate was used to prepare ten-fold dilutions up to 10⁻⁷ with peptone water [23]. Total viable bacterial count (TVBC): Drop method was used to inoculate agar plates. Aerobic mesophiles were determined using Plate Count Agar (Oxoid CM 325), plates were incubated at 30 °C, for 24 to 48 hours. Total Enterobacteriaceae count (TEC): Enterobacteriaceae were isolated and enumerated on violet red bile glucose agar. Plates were incubated at 37 °C, for 24 to 48 hours. Pink-red colonies with precipitation were taken into consideration.

Total coliform count (TCC) and *Escherichia coli*: Coliform and *Escherichia coli* were enumerated on MacConkey agar and eosin methylene blue (EMB) agar, respectively. Plates were incubated at 37 °C, for 24-48 hours. Pink red colonies with precipitation on MacConkey agar were enumerated as coliforms while colonies with greenish metallic sheen on EMB agar were counted as *E. coli*. Indole, methyl red, Voges-Proskauer and Citrate (IMViC) tests were performed on colonies that showed shiny-metallic green to identify *E. coli*. Enterococci spp were counted on Slanetz Bartley medium after incubating aerobically at 37 °C, for 24-48 hours. The red colonies grown on this medium were taken into considerations.

Total *Staphylococcus aureus* count and micrococci: *Staphylococcus aureus* and micrococci were enumerated on Mannitol salt agar (MSA) and Baird Parker agar, respectively. Plates were incubated at 37 °C, for 24 to 48 hours. Yellow colonies on MSA were regarded

as *Staphylococcus aureus* while small brown-black colonies without zones on Baird Parker agar were considered as *Micrococcus* spp. Catalase and coagulase tests were used for identification of *Staphylococcus aureus* [24].

Bacillus cereus: *B. cereus* was isolated on Mannitol egg-yolk polymyxin (MYP) agar aerobically at 30 °C, for 24 to 48 hours. Typical colonies of *Bacillus cereus* are rough and dry with a bright pink background surrounded by an egg yolk precipitate. *Salmonella* spp: A twenty-five-gram sample was incubated in 225 ml buffered peptone water at 37 °C, for 24 hours. Subsequently, 0.1ml was inoculated into Rappaport Vassiliadis Broth and incubated at 43 °C, for 24 to 48 hours. Streak plates were prepared on *Salmonella-Shigella* agar and incubated at 24 to 48 hours. Pink-red colonies with black centers were inoculated onto triple sugar iron agar and lysin iron agar. Biochemical tests were performed for the identification of *Salmonella* spp. Biochemical characterization of isolates was carried out according to standard procedures [25].

Statistical Analysis

Data collated were analyzed using MedCalc statistical software version 17.2 (a statistical software package designed for the biomedical sciences). Simple means, percentages and frequencies from different locations were computed. These were compared using one-way Analysis of Variance (ANOVA) and independent t- test.

Results

Table 1 showed the standard microbial load specification in animal food products. This was necessary to categorize the bacteriological quality of the rabbit meat samples into grades I, II and III in order to determine whether sample is satisfactory, passable or unsatisfactory, respectively.

Grades	TVBC (total viable count)/g at 30 °C,	Description
I	<1/2 million	Satisfactory
II	1/2 million to <10 million	Passable
III	10 million and more	Unsatisfactory

Source: Williams, *et al.* [22].

Table 1: Standard Microbial Load Specification in Animal Food Products

Table 2 showed the bacteriological quality of smoked rabbit meat sold on Lagos-Benin expressway, Nigeria. Results revealed that total viable bacteria count (TVBC) ranged from 1.48×10^5 to 3.20×10^5 CFU/g as obtained in sample 6 and sample 1, respectively. The total Enterobacteriaceae count (TEC) ranged from 1.05×10^3 CFU/g (in sample 1) to 1.36×10^3 CFU/g (in sample 9). Total coliform count (TCC) ranged from 1.10×10^3 to 1.44×10^3 CFU/g as obtained in sample 9 and sample 3, respectively, and total *Staphylococcus aureus* count (TSAC) ranged from 1.14×10^2 to 2.36×10^2 CFU/g as in samples 1 and 4, respectively.

Location/ Sample	Bacterial counts in CFU/g				
	TVBC	TEC	TCC	TSAC	<i>Salmonella</i> count
1	3.20×10^{5a}	1.05×10^{3a}	1.44×10^{3b}	1.22×10^{2a}	1.4×10^{3a}
2	3.00×10^{5a}	1.20×10^{3a}	1.23×10^{3a}	1.17×10^{2a}	-
3	1.83×10^{5b}	1.25×10^{3a}	1.42×10^{3b}	1.62×10^{2a}	-
4	2.00×10^{5b}	1.32×10^{3b}	1.14×10^{3a}	1.19×10^{2a}	2.0×10^{3b}
5	2.94×10^{5a}	1.22×10^{3a}	1.10×10^{3a}	1.20×10^{2a}	-
6	1.48×10^{5b}	1.23×10^{3b}	1.28×10^{3b}	1.33×10^{2a}	-
7	2.24×10^{5b}	1.15×10^{3a}	1.30×10^{3b}	1.14×10^{2a}	-
8	2.47×10^{5b}	1.12×10^{3a}	1.25×10^{3b}	2.36×10^{2b}	-
9	3.00×10^{5a}	1.36×10^{3b}	1.21×10^{3a}	2.10×10^{2b}	-
10	2.35×10^{5b}	1.28×10^{3b}	1.33×10^{3b}	1.20×10^{2a}	-

Keys:

TVBC: Total viable bacterial count; TEC: Total Enterobacteriaceae count

TCC: Total coliform count; TSAC: Total *Staphylococcus aureus* count

Values with same superscript along same column showed no significant difference ($P > 0.05$).

Table 2: Bacteriological Quality of Rabbit Meat sold on Lagos-Benin Expressway, Nigeria

Table 3 showed the distribution of bacterial isolates from rabbit meat sold on Lagos-Benin expressway, Nigeria. Each sample was found to contribute to the bacterial diversity encountered in this study; the occurrence of bacterial species varied in the different samples. Figure 1 shows the percentage occurrence of bacterial species from rabbit meat on sale in Lagos-Benin Expressway, Nigeria. *Staphylococcus aureus* had the highest percentage frequency of 30.3%; this was followed by *Klebsiella* spp and *Micrococcus* spp with the same percentage occurrence of 15.2%. *Escherichia coli* and *Enterococcus* spp had percentage occurrences of 12.1% and 9.1%, respectively. However, *Salmonella* spp, *Pseudomonas aeruginosa* and *Proteus* spp had same percentage occurrence of 6.1%.

Sample	Bacterial Isolates
1	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Pseudomonas aeruginosa</i>
2	<i>Klebsiella</i> spp, <i>Enterococcus</i> spp , <i>Staphylococcus aureus</i> <i>Proteus</i> spp
3	<i>Proteus</i> spp, <i>Micrococcus</i> spp, <i>Staphylococcus aureus</i>
4	<i>Staphylococcus aureus</i> , <i>Klebsiella</i> spp, <i>Salmonella</i> spp,
5	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> spp,
6	<i>Staphylococcus aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> spp,
7	<i>Micrococcus</i> spp, <i>Staphylococcus aureus</i> , <i>Klebsiella</i> spp.
8	<i>Staphylococcus aureus</i> , <i>Micrococcus</i> spp, <i>E. coli</i>
9	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella</i> spp, <i>Micrococcus</i> spp
10	<i>Staphylococcus aureus</i> , <i>Micrococcus</i> spp, <i>E. coli</i> , <i>Enterococcus</i> spp

Table 3: Occurrence of Bacterial Species in Rabbit Meat sold on Lagos-Benin Expressway, Nigeria

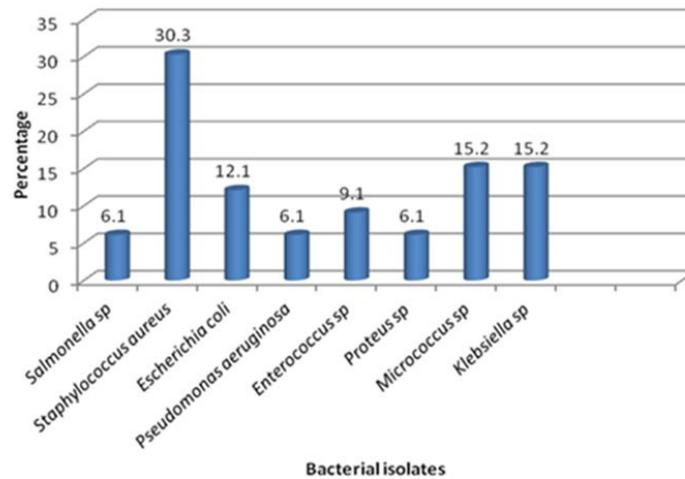


Figure 1: Percentage occurrence of bacteria isolated from smoked rabbit meat sold on Lagos-Benin Expressway

Test	<i>S. aureus</i>	<i>Salmonella</i> spp	<i>Micrococcus</i> spp	<i>Enterococcus</i> spp	<i>E. coli</i>	<i>Proteus</i> spp	<i>Klebsiella</i> spp	<i>P. auroginosa</i>
Gram's reaction	+	-	+	+	-	-	-	-
Catalase	+	-	+	-	+	-	+	+
Coagulase	+	-	-	+	-	-	-	-
Citrate Utilization	-	-	+	+	+	+	+	+
Oxidase	-	-	-	+	-	-	+	+
Urease	+	-	-	-	-	+	+	-
Indole	-	-	-	-	+	+	-	-
Glucose	+	+	+	+	+	+	+	-
Lactose	+	+	-	+	+	+	+	-
Sucrose	+	-	+	+	+	+	+	-
Mannitol	+	+	-	+	+	-	-	+
Maltose	+	+	+	+	+	+	-	-
Cellular morphology	Cocci	Rod	Cocci	Cocci	Rod	Rod	Rod	Rod
Growth on Mannitol salt agar	Bright yellow	ND	Pink	ND	ND	ND	ND	ND
Growth on MacConkey agar	N/A	Colourless	N/A	Pink	Red/Pink	Colourless	Mucoid	Pale
Growth on blood agar	Creamy white	Smooth white	Smooth pigmented	Creamy	Circular	Swarming waves	Large white	Greenish

Keys: + = Positive; - = Negative; N/A = Not applicable; ND = Not determined.

Table 4: Biochemical characteristics of bacterial isolates from rabbit meat sold on Lagos-Benin expressway, Nigeria

Bacterial isolates were *Staphylococcus aureus*, *Klebsiella* spp, *Micrococcus* spp, *Escherichia coli*, *Enterococcus* spp, *Salmonella* spp, *Pseudomonas aeruginosa* and *Proteus* spp.

Values obtained from the ten different locations were analysed statistically to establish if there was a significant difference among the data obtained from the different locations, and hence the relationship. There was no statistical difference in TVBC obtained from locations 1, 2, 5 and 9; data obtained from locations 3, 4, 6, 7, 8 and 10 were also not statistically different ($P > 0.05$). However, the data obtained from former set of locations showed significant difference from the latter ($P < 0.05$).

Discussion and Conclusions

Staphylococcus aureus, *Klebsiella* spp, *Micrococcus* spp, *Escherichia coli*, *Enterococcus* spp, *Salmonella* spp, *Pseudomonas aeruginosa* and *Proteus* spp were encountered in this study. Some of these bacteria had been earlier found in foods, water, environment and other places [26-30]. The presence of these organisms in rabbit meats depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering, processing and packaging of product.

Although the total viable bacteria count (TVBC) obtained in this study ranged from 1.48×10^5 to 3.20×10^5 CFU/g indicating a satisfactory quality according to the standard microbial load specification in animal food products, 2002), the bacteriological quality of the product is still regarded as poor from bacteriological standing point as it did fall within the standards of other bacteriological parameters for determination of animal food quality (Table 1) [21]. The TEC ranged from 1.05×10^3 CFU/g to 1.36×10^3 CFU/g, TCC ranged from 1.10×10^3 to 1.44×10^3 CFU/g and TSAC ranged from 1.14×10^2 to 2.36×10^2 CFU/g; the presence of these group of indicator organisms and other potential pathogens such as *Staphylococcus aureus* and *Shigella* spp is sufficient to pose public health threat.

Presence of these potential pathogens might be due to possible contamination during sales or unhygienic handling of the meats right from the slaughtering, butchering plants and processing or due to contamination from the skin, mouth, or nose of the handlers. *Staphylococcus aureus* could have been introduced directly into foods by process line workers, or on hands and arms coming into contact with the food, or by coughing and sneezing [27,31]. The presence of potential pathogens such as *Klebsiella* spp, and *E. coli* among others, encountered in this study is public health concern. The consequence of their presence in rabbit meat indicates public health hazard and give warning signal for the possible occurrence of food borne intoxication [32].

The isolation of *Enterococcus* spp may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering, because *Enterococcus* spp are also inhabitants of dairy products [30,33]. *Salmonella* spp encountered in this study is also a pathogenic organism of public health significance, and of practical impact. This organism might have contaminated the meats during the process of handling by sellers. The presence of the indicator and other organisms is of special concern and perhaps the greatest danger associated with meats used for food preparation and eating purposes. This is indicative of the fact that the food product has been contaminated with matter of faecal origin [34]. It demonstrates a potential health risk as the organisms are pathogenic and cause complications in children [35].

This result agrees with previous report by Bello, *et al.* that foods of animal origin either cooked or uncooked were predominantly contaminated with enteric bacteria [36]. Waites and Arbuthnott also reported 50% *E. coli* contamination in minced meat, sausage rolls and pies. This is also in accordance with the assertion of Bello and Osho that improper handling and improper hygiene might lead to the contamination of ready-to-eat foods and this might eventually affect the health of the consumers [37,38]. Thus, street-vended rabbit meat is a viable source of potential pathogens. Some of these diseases could spread and acquire epidemic status which poses serious health hazards.

Bacteria isolated from rabbit meat samples in this study could have been as a result of the relatively complex series of interrelated steps designed to convert rabbits into ready-to-cook whole carcasses, cut-up carcass parts, or various forms of deboned meat products which might have given rooms to a certain level of contamination to occur. Therefore, cautions and improvement on hygienic practices are necessary as some potential pathogenic organisms and members of coliforms encountered in this study could pose public health threat. However, the processors/handlers/sellers should observe strict hygienic measures so that they do not serve as source of chance inoculation of microorganisms and faecal contamination of rabbit meats and other food products of animal origin. Thorough cooking of rabbit meats and other bush meats is also crucial.

By the way, it has been underlined that transport and handling before slaughter are of utmost importance for product and meat quality. Good manufacturing practices include benefits that are in the interest of every party involved: the farmer, the slaughterhouse, the consumer and the public. Rabbit product quality can be considerably improved through respecting a recommended feed withdrawal period and holding time prior to slaughter. Additionally, further studies on slaughter and post slaughter handling techniques such as stunning, chilling, electrical stimulation, deboning to examine their influence on rabbit meat quality and stability are needed.

It was revealed from this study that rabbit meats sold on Lagos-Benin Expressway, Nigeria are of satisfactory quality from bacteriological point of view. Though, from bacteriological point view, the total viable bacterial counts in samples which fell within grade I (satisfactory) in accordance with the standard microbial load specification in animal food products, but the presence of potential pathogens in these samples poses serious public health risk.

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