

## **Mycoflora and Heavy Metals in Roasted Cow Meat (Suya) From Some Taxi Terminals in Port Harcourt**

EMELIKE, N.J.T. & ACHINEWHU, S.C.  
Rivers State University, Port Harcourt, Nigeria

**ABSTRACT** The impact of automobile exhaust on the levels of heavy metals accumulation and associated mycoflora of roasted cow meat (suya) in ten taxi terminals in Port Harcourt were assessed. This was done to ascertain the safety of consumption of roasted cow meat sold in the motor parks and other taxi terminals in Port Harcourt and the associated health risk. A total of 20 sticks of roasted meat (suya) samples were purchased from the vendors, 2 sticks of roasted cow meat from each location for the ten locations and exposed for two days at the various terminals clearly labelled. The samples were later analysed in the Food Science and Technology Laboratory with the atomic absorption Spectro-photometer in the Rivers State University, Port Harcourt. From the investigation carried out, ten heavy metals comprising Antimony, Cadmium, Chromium, Lead, Mercury, Nickel, Copper, Cobalt, Manganese and Zinc were detected. The concentration of Antimony ranged from (0.006 to 0.008/ 1000g), Cadmium (0.07ug to 0 0.08/ 1000g), Chromium (0.02 to 0.03/1000g), Lead (0.25 to 0.26 ug/1000g), Mercury (0.06 to 0.07 ug/1000g), Nickel (1.25 to 1.26 ug/ 1000g), Copper (35.5 to35.6 ug/ 1000g), Cobalt (0.05 to 0.053 ug/ 1000g), Manganese (25.2 to 25.5 ug/ 1000g) and Zinc (250-252ug/ 1000g). These levels are within the naturally occurring levels in animal products and therefore accepted for consumption without causing any toxicity in man. However, the consumption of these products for a long period of time will certainly become toxic because heavy metals are known to bioaccumulation. A total of nine fungi were isolated from the different taxi terminals with varying degrees of incidence and include *Neurospora crassa*, *Aspergillus niger*, *Aspergillus tamari*, *Penicillium italicum*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Mucor mucedo*, *Geotrichum candidum* and *Rhizopus stolonifer*. *Aspergillus tamari* occurred most in six locations followed by *Fusarium oxysporum* which occurred in five locations with *Fusarium moniliforme* occurring only in one location. Roasted cow meat samples from Gambia Street and Azikiwe taxi terminals recorded six fungi respectively, while the least number of fungi was isolated from samples from Okija taxi terminal. All the fungal isolates caused soft rot of the roasted cow meat an indication of pathogenicity and is of great health concern.

**Keywords:** Heavy metals, mycoflora, roasted cow meat, taxi terminals.

### **Introductions**

Heavy metals are stable pollutants which cannot be degraded or destroyed. They are members of an ill- defined subset of elements that exhibit metallic properties which

include mainly the transition metals. They are made up of metals with high atomic weights and are easily distributed from either natural or industrial sources and eventually can be incorporated to natural sources of food like meat. Heavy metals can damage living things at low concentrations and are bio-accumulating in food chain. (Tanee and Kinako, 2008). They are metals of high molecular weight that are of concern because they are generally toxic to animal life and human health if naturally occurring concentrations are exceeded, e.g. Arsenic, Chromium, Lead and Mercury (Tanee and Kinako, 2008). Heavy metals can arise from many sources but most commonly from the purification of metals such as the smelting of copper and the preparation of nuclear fuels (Turner and Leytem, 2004). However, chronic exposure may result from contaminated foods, air, water or dust. Living near a hazardous dump site, spending time in areas with deteriorating lead paint; maternal transfer in the womb (Robert, 1999); or from participating in hobbies that use lead paints or solder. Chronic exposure may occur in either the home or workplace. Symptoms of chronic toxicity are often similar to many common conditions and may not be readily recognized (U.S.E.P.A, 2001). Routes of exposure include inhalation, skin or eye contact, and ingestion (ATSDR MMGs and ToxFAQS; Anon, 1993, WHO, 1998; International Occupational Safety and Health Information Centre; 1999; Roberts, 1999; Dupler 2001; Ferner, 2001). Different heavy metals have different reactions in man when consumed in large quantity. Toxic effects of lead are represented in such symptoms as abdominal pains, convulsions, hypertension, renal dysfunctions, and loss of appetite, fatigue, sleeplessness, numbness, arthritis, hallucinations, headaches and several other birth defects (Zayas *et al*, 1995). Mercury toxicity may result in permanent damage of the nervous system and kidney (Ewan *et al*, 1996), retardation in learning in children (O'Brien, 2001). Symptoms of other heavy metal toxicity in man and their associated negative effects are well documented (WHO, 1998). Roasted cow meat consumption locally referred to as (Suya) in Nigeria is a cherished delicacy. The cow meat which is usually prepared by the Hausa men are hawked and sold in most motor parks and people consume this meat as snacks. Sometimes the roasted meat is not sold off immediately they are prepared, as some of them are prepared and kept for several days and warmed on fire whenever customers come to buy them. These products attract flies which may contaminate them and also exposed to unhygienic conditions which increase their microbial contamination (Okaka, 1997). The fact that these roasted meat vendors also sell their products at motor parks further expose these products to heavy metal contamination. Fungi are among the most serious microbes that cause serious deterioration of plants and animal products (Onuegbu, 2002). This is because fungi are found in almost everywhere in the environment (Chuku and Chuku, 2015). The problems associated with the consumption of fungal contaminated vegetables, fruits, nuts and animal products cannot be over emphasized. Fungi cause various health problems ranging from minor illnesses to chronic and deadly diseases such as cancers resulting from the consumptions of fungi contaminated products. The consumption of contaminated groundnut bio-accumulates *Aspergillus flavus* which causes cancer in man (Alexopolous, 1977). This research therefore investigated the fungal contamination of roasted cow meat and also assessed the levels of heavy metals accumulation of roasted cow meat exposed to car exhaust at different taxis terminals in Port Harcourt

the knowledge of which will expose the danger associated with the consumption of roasted meat sold in exposed places and will proffer solutions to these problems.

### **Materials and Methods**

Roasted cow meat samples (Suya) were bought from ten different taxi terminals in Port Harcourt. The locations were Ikwerre taxi terminal, Abuja lane, Rumuokoro junction, Okija, Egede, Gambia Street, Ojoto, Azikiwe, Billy close and mile 111 market junction. Ten sticks of roasted cow meat were purchased from each of the locations and tied up in newspaper and labelled accordingly. The roasted cow meat was transported to the Department of Applied and Environmental Biology for further studies.

#### *Heavy metals assessment in roasted cow meat*

Three sticks of the roasted cow meat from the different locations were taken to the Department of Food Science and Technology for heavy metals determination. The method used was the AOAC (2005) methods of analysis.

#### *Preparation of mycological medium*

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120°C for an hour after washing with soap, while other equipment was surface sterilized with 70% ethanol to reduce microbial contamination (Agrios, 2005). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminium foil. The conical flask containing the mycological medium was autoclaved at 121° C and pressure of 1.1kg cm<sup>-3</sup> for 15 minutes. The molten agar was allowed to cool to about 40 ° C and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

#### *Isolation of fungi from roasted cow meat*

Tin slices of roasted cow meat samples showing visible signs of spoilage by moulds from the different locations were washed in tap water, rinsed in distilled water and surface sterilized with 70% ethanol and labelled accordingly. The samples were inoculated onto Sabouraud Dextrose Agar (SDA) in Petri dishes onto which ampicillin was added to hinder the growth of bacteria in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25° C ± 3° C (Baudoni, 1988). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates was obtained after a series of isolations.

### *Identification of fungal organisms from roasted cow meat*

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high-power objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Samson *et al*, 1981, Olds, 1983, Barnett and Hunter, 1972).

### *Pathogenicity studies*

Pathogenicity studies was carried out on freshly roasted cow meat to check if the fungi isolated from moulded roasted cow meat can cause spoilage of the freshly prepared roasted cow meat samples. The methods of (Agrios, 2005, and Trigiano, 2004) was basically followed. Roasted cow meat samples from the ten locations were surface sterilized with 70% ethyl alcohol and each of the fungal isolates was aseptically inoculated onto the roasted cow meat from their respective locations and placed on SDA in Petri dishes. The set up was monitored regularly for growth at room temperature of  $25^{\circ}\text{C}\pm 3^{\circ}\text{C}$  for 7 days.

### *Heavy metal assessment in roasted cow meat (Suya)*

Roasted cow meat purchased from the ten different taxi terminals were exposed for 24 hours prior to the assessment of heavy metals in them. The exposed samples of roasted cow meat were taken to the Food Science and Technology for heavy metals determination. The Atomic Absorption Spectrophotometric method of Pearson (1976) was used.

### **Statistical analysis**

The data generated from this study were interpreted with the appropriate statistics such as percentages, means and standard errors. The use of these statistical methods is among the appropriate for the complete randomised experiments as was used in this work.

### **Results**

#### *Fungal isolates from roasted cow meat from the various taxis terminals*

Results of the fungal isolates from roasted cow meat from ten different locations in Port Harcourt are presented in Table 1. Different fungi with varying degrees of incidence were isolated and identified. Roasted cow meat from Gambia Street and Azikiwe taxi terminals harboured the highest number of fungi. These locations recorded six fungi each. Roasted cow meat samples from Mile 111 market junction terminal recorded five fungi while roasted cow meat samples from Ojoto, Billy close, Ikwerre road, Rumuokoro and Abuja taxi terminals recorded three fungi respectively. The

least number of one fungus was isolated from roasted cow meat from Okija lane taxi terminal while roasted cow meat samples from Egede Street had only two fungi.

*Table 1: Fungal isolates from roasted cow meat (Suya) from different locations in Port Harcourt.*

Location	Fungal isolates	Incidence (%)
Gambia Street	<i>Neurospora crassa</i>	20±0.01
	<i>Aspergillus niger</i>	30±0.011
	<i>Aspergillus tamari</i>	50±0.02
	<i>Penicillium italicum</i>	30±0.10
	<i>Fusarium oxysporum</i>	30±0.21
	<i>Muccor mucedo</i>	50±0.22
Okija	<i>Geotrichum candidum</i>	60 ±0.04
Ojoto	<i>Rhizopus stolonifer</i>	60±0.03
	<i>Aspergillus tamari</i>	80±0.11
	<i>Fusarium oxysporum</i>	30±0.03
Abuja lane	<i>Geotrichum candidum</i>	70±0.04
	<i>Fusarium oxysporum</i>	10±0.02
	<i>Aspergillus tamari</i>	40±0.11
Rumuokoro	<i>Aspergillus tamari</i>	50±0.03
	<i>Penicillium italicum</i>	20±0.01
	<i>Fusarium oxysporum</i>	30±0.01
Egede street	<i>Geotrichum candidum</i>	10±0.02
	<i>Linderina</i>	60±0.05
Ikwerre road	<i>Neurospora crassa</i>	30±0.04
	<i>Muccor mucedo</i>	40±0.22
	<i>Aspergillus tamari</i>	20±0.01
Billy close	<i>Muccor mucedo</i>	50±0.04
	<i>Fusarium oxysporum</i>	70±0.02
	<i>Sclerotium rolfsii</i>	30±0.01
Azikiwe	<i>A. tamari</i>	60±0.05
	<i>A. niger</i>	30±0.03
	<i>P. italicum</i>	20±0.01
	<i>F. oxysporium</i>	20±0.04
	<i>R. stolonifer</i>	20±0.02
	<i>G. candidum</i>	40±0.01
Market junction	<i>R. stolonifer</i>	60±0.12
	<i>A. tamari</i>	10±0.33
	<i>Pen. italicum</i>	5±0.21
	<i>G. candidum</i>	30±0.10
	<i>A. niger</i>	10±0.11

*Determination of heavy metals in roasted cow meat (Suya)*

Results of heavy metals assessment of the roasted cow meat (Suya) from the ten locations are presented in Table 2. Ten heavy metals were identified in roasted cow meat with variable concentrations. The levels of Antimony accumulated in roasted cow meat ranged from (0.005 to 0.009) in the various roasted cow meat samples. Cadmium varied from (0.01 to 0.10), Chromium ranged from (0.03 to 0.015), Lead ranged from (0.21 to 0.35), Mercury ranged from (0.06 to 0.09), Nickel from (1.12 to 1.35), Copper from (35.5 to 42.5), Cobalt from (0.03 to 0.06), Manganese from (24.5 to 26.5) and Zinc from (200 to 300mg/1000g).

*Table 2: Concentrations of heavy metals in roasted cow meat from different taxi terminals in Port Harcourt.*

Pollutants as heavy metal (mg/1000g)										
Locations	Anti-mony	Cad-mium	Chro-mium	Le-ad	Mer-cury	Nic-kel	Cop-per	Co-balt	Man-ganese	Zi-nc
Ikwerre road	0.008	0.07	0.03	0.25	0.06	1.25	35.5	0.05	25.2	250
Abuja lane	0.009	0.08	0.05	0.21	0.06	1.30	36.1	0.04	26.5	300
Rumuokoro	0.007	0.10	0.04	0.22	0.056	1.26	37.0	0.05	25.6	300
Okija	0.008	0.08	0.03	0.35	0.07	1.12	35.6	0.04	26.00	200
Egede	0.007	0.09	0.07	0.30	0.06	1.15	35.5	0.05	25.12	200
Gambia	0.006	0.07	0.04	0.32	0.07	1.14	36.8	0.03	25.20	230
Ojoto	0.005	0.06	0.11	0.25	0.08	1.30	40.5	0.06	25.10	240
Azikiwe	0.006	0.012	0.15	0.27	0.09	1.35	42.6	0.05	25.20	205
Billy close	0.009	0.09	0.08	0.22	0.07	1.25	40.5	0.025	26.4	260
Market-junction	0.008	0.08	0.09	0.26	0.06	1.27	38.5	0.03	24.5	250

**Discussion**

Heavy metals in plants and animals can be hazardous when consumed in large quantities (Collins, 2007). They are bio-accumulating in nature and very difficult to get out of the environment. The dumping of hazardous wastes into dump sites, rivers and lakes have led to pollution of the environment and marine environment resulting in terminal diseases like cancer and several other diseases. Marine lives are destroyed and water bodies polluted (EPA, 2010). Most processed and unprocessed animal and plant materials are easily contaminated by heavy metals when exposed to such automobile exhaust, and other hydrocarbons. These hazardous materials are absorbed into the tissues of these products and magnify (Emem, 2009). The accumulation of ten heavy metals in roasted cow meat from various when exposed to car exhaust in taxi terminals is pioneering since there are dearth of information on this work. Heavy metals are known to constitute serious health problems when consumed in large amount (WHO, 1998). However, it was observed from the study that the limit of the various heavy metals accumulated in roasted cow meat were within the acceptable limits that could be tolerated in man although when consumed for a long period of time will bio-magnify and will eventually become very dangerous to man.

Heavy metal toxicity in man has been reported to cause kidney failure, cardiac irregularities, breathing difficulties, skin irritation and general malfunctioning of vital organs in the body (Anon, 2001, James, 2001). The toxic effects of heavy metals such as lead, aluminium, chromium, copper and zinc have been reported by early researchers (Ferner, 2001, Joseph, 2001 and Anon, 2001). However, there are suggestions on some natural remedies for their detoxification in man such as chelation (Ferner, 2001, James, 2001), gastric lavage (Ferner, 2001), whole bowel irrigation (Ferner, 2001, James, 2001), emesis (Anon, 2001), cathartic (Goyer, 1996), fluid dialysis and drugs treatment (Anon, 2001, James, 2001). The problems associated with the consumption of fungal contaminated plants and animal products are well documented (Alexopolous, 1977). Fungi because of their high level of proliferations in a wide range of environment are known to be responsible for the deterioration of most agricultural products both in the field and store (Onuegbu, 2002). Fungi are known to secrete enzymes such as polygalacturonase and cellulose enzymes which help in the quick deterioration of several food materials (Onuegbu, 1999, Chuku *et al.*, 2005). These organisms also harbour mycotoxins which cause cancers in man.

### **Conclusion**

The results from this research have shown that roasted cow meat (Suya) sold in taxi terminals accumulate heavy metals which when consumed in large quantity can cause serious health challenge. In recent times, it is quite imperative that diseases of unknown sources have emanated and have reduced the life span of the populace which in most cases have been attributed to witchcraft agents. It is important to know that we are sick of what we consume ignorantly and as such food items should be purchased from designated shops free from contaminants. It was also observed that these roasted cow meat harbour numerous fungi which further make their consumption unfit for man considering the associated dangerous problems fungi cause in man. Fungi secrete mycotoxins which cause cancers in man, and cancers are silent killers. It is hereby advised that fungal contaminated foods should not be consumed. Proper location of the sites where roasted cow meat (Suya) is sold will reduce the danger of fungal and heavy metals contamination of this cherished food.

### **Recommendations**

Avoid smoking, eating and drinking in work places particularly in industries where products that emit heavy metal are used. The government should ensure that roasted cow meat vendors are assigned special sites free from pollution from car exhaust emission and other hazardous elements to safe guard the health of the citizens. Roasted cow meat and other food materials should be properly handled to avoid their being contaminated. Awareness must be created in homes and the society at large concerning heavy metals poisoning.

### **Correspondence**

Emelike, N.J.T & Achinewhu, S.C  
Department of Food Science and Technology  
Rivers State University, Port Harcourt, Nigeria

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