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CHEMICAL AND MICROBIAL SAFETY OF STREET VENDED EGG ROLL IN ABEOKUTA METROPOLIS

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ABSTRACT

A total of 12 samples of egg rolls retailed in three locations in Abeokuta and a laboratory prepared sample serving as control were analyzed for their microbial load and presence of heavy metals (Lead, Cadmium, Chromium). The samples were analyzed for bacteria using standard procedures. Analysis of the food samples showed that total viable bacterial count ranged from 1.2 x 10^5 to 4.2 x 10^5 cfu/g, total staphylococcus count ranged between 1.2 x 10^4 and 6.4 x 10^4 cfu/g, coliform count ranged from 1.0 x 10^4 to 3.0 x 10^4cfu/g, total campylobacter count ranged between 1.0x10^4 and1.3x10^4 cfu/g and there were no growths for the total listeria count. Five bacteria genera were isolated including *Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes, Salmonella typhi, Campylobacter coli.* Cadmium and Chromium contamination ranged from 3.50±0.71 - 12.50±0.71 and 0.75±0.35 - 3.50±0.00 respectively. Statistical analysis of the heavy metals shows a significant difference ($p<0.05$) between the control and samples. The level of microbial contamination could be associated with inadequate handling and processing by processors and vendors, contamination caused by either poor hygiene or poor quality of water used. Moreover, contamination of cadmium and chromium in all the samples could be incident upon the water used in producing it, however lead was absent in all the samples. It is therefore recommended that health education should be done for food handlers and consumers about food safety.

Keywords: Street vended egg roll, microbial safety, heavy metals, analysis

INTRODUCTION

In the past, people used to prepare and consume fresh foods at home. Now the trends are changing as the people are trying to keep up with the hectic routines of busy life. The food sector is evolving accordingly to keep up with the need of the time and many ready-to-eat products are offered and sold on the streets. Among these ready-to-eat food product, snacks offer convenience and satisfaction at the same time for the busy and health conscious customers (Musaiger *et al*., 2007). These snacks as compared to the perishable food items are not limited to bakeries only, rather they are distributed over wide area from manufacturing points.

Snacks sold by street vendors are widely consumed by thousands of people. These snacks are ready-to-eat foods prepared and sold by vendors and hawkers especially in the street

and other similar public places like motor parks (FAO, 1997).

The main raw materials are wheat flour, sugar, eggs, yeast and milk cream. Eggroll being a pastry with egg filling is an excellent growth medium for many kinds of microorganisms, as it provides rich nutrient for microorganisms. Freshly produced egg rolls would be sterile and not contain viable microorganisms but soon become contaminated upon exposure to air, surfaces, unhygienic handling conditions and transportation. In addition, these microbes play major roles in the safety and spoilage of these snacks. These snacks are served after passing through long chain of steps involved in production, processing, distribution and marketing. The major contaminants likely to be present in these snacks are *Coliform, Escherichia coli, Staphylococcus* and *Salmonella*. Salmonella is a well-known foodborne infectious agent that account for major share as the causative agent of food borne illnesses (Thorns, 2000). The lack of hygienic measures leads to the introduction of *staphylococcus, coliforms* and *E coli* (M'hir *et al*., 2007). The handlers and vendors themselves can be sources of contamination as presence of staphylococcus on the food handlers is well known (Acco *et al*., 2003) and if products are handled without observing food safety principles, it contaminates the product with staphylococcus. *Staphylococcus aureus* is considered the third most important cause of diseases in the world amongst the reported food-borne illness (Zhang *et al*., 1998).

Among coliform, *Escherichia coli* is considered to be the most important pathogen that causes food borne illness. The organism is known to cause large outbreaks originating with the consumption of contaminated food. Illness caused *by E.coli* infection can range from self-limited watery diarrhoea to life-threatening manifestations such as haemolytic uremic syndrome or thrombotic thrombocytopenic purpura (Padhye and Doyle, 1992).

Heavy metals have been reported to have positive and negative roles in human life, some like iron, zinc and copper are essential for biochemical reactions in the body while others like cadmium, lead and arsenic are major contaminants of food supply and may be considered the most important challenge to our environment (Zaidi *et al*., 2005).

The consumption of street vended foods has been suggested to potentially increase the risk of food borne diseases as they are readily contaminated from different sources and may not be safe for consumers. Evaluating the quality and microbiological safety of these snacks provides an insight on the safety of our street vended snacks, indicating to us the quality of our daily consumable snacks and whether they are microbiologically safe or not. It is therefore crucial that eggrolls are upgraded for quality and safety in order to safeguard the consumers from consumption of poorly prepared and low quality street food. Hence this study focused on determining the level of heavy metals in street vended egg rolls and to evaluate the incidence of coliform, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp* and observing the overall microbial load in the egg roll snack available in Abeokuta metropolis from vendors and processors.

MATERIALS AND METHODS

Source of sample

Egg roll samples were purchased from three processing and vending locations (Kuto, Lafenwa and Obantoko) in Abeokuta, Ogun state, Nigeria

Sample collection

A total of 12 samples were collected comprising 1 sample from three processors in the locations and from 3 vendors from each processor and a laboratory sample used as control. Each sample was collected aseptically, packaged separately in a sterile polythene bag and transported to the laboratory for analysis within 2 h of collection.

Production of laboratory egg roll (control)

The production of egg roll follows the basic step of mixing various ingredients required for the production of egg roll, following the same method used by street food processors. Figure 1 shows a flowchart of the egg roll production process.

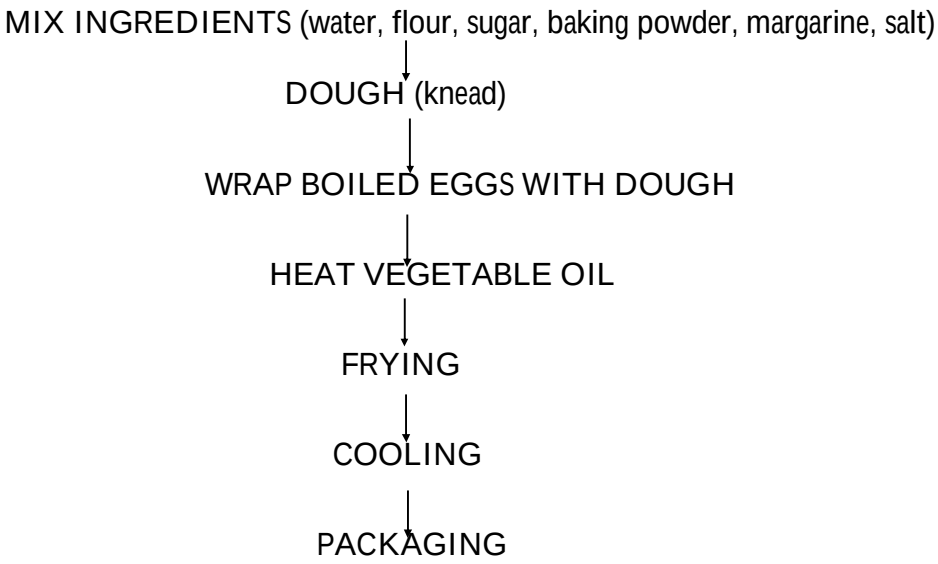

Figure 1: Flow chart for the production of street vended egg roll (observation 2015)

MICROBIOLOGICAL ANALYSIS

Microbiological analysis included enumeration, characterization and identification of potential pathogens according to standard procedures (AOAC, 2012) for the number of bacteria including *Staphylococcus aureus, Salmonella, Listeria, Campylobacter* and *Coliforms.*

CHEMICAL ANALYSIS

Chemical analysis included the enumeration of the level of lead, chromium and cadmium heavy metals in the egg roll samples

MATERIALS

High purity of certified reagents was used for all analysis. Concentrated Trioxonitrate (V) acid (HNO_3) was used for digestion while distilled water was used to rinse the apparatus before and after every point of use and deionized water to analyze the digested samples.

Ashing of Sample

Blended sample (2g) was weighed in a tarred silica dish and was transferred to a temperature controlled muffle furnace, the muffle was kept at 300 ^oC. After the material was

dried and charred, the temperature was allowed to rise to 450 ^{o}C and ashed at this temperature.

After complete ashing, the dish was removed from the muffle furnace, cooled and covered with glass (AOAC, 2012).

Sample digestion for Atomic Absorption determination

Two grams (2g) of each food sample was weighed into a 100 ml digestion flask. Exactly 15 ml of concentrated trioxonitrate (V) acid (HNO_3) was added. The digestion flask containing the sample was then placed on a bath (Gerhardt) in a fume cupboard, and sufficiently heated until a clear solution was obtained (AOAC, 2012).

Atomic Absorption Analysis of Digested Samples

After digestion, each of digested samples was carefully poured into a 100 ml volumetric flask and made up to the mark. The metal elements (Lead, Cadmium and Chromium) were analyzed by using Atomic Absorption Spectrophotometer (Thermoscientific S series, S4AA system) at wavelength of 217.0nm, 228.8nm and 193.7nm respectively using acetylene gas, air and N_2gas (AOAC, 2012).

Statistical Analysis

All analysis was reported as means of two replicates. Data analysis was carried out using one-way analysis of variance (ANOVA) based on Completely Randomized Design (CRD) using Statistical Package for Social Sciences (SPSS) version 16.0 Mean separation was by Duncan's New Multiple Range Test (DNMRT) and significance accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the mean total colony forming count for total viable count, *Listeria* count, *S. aureus,* Coliform count and *Campylobacter* count per gram of Egg roll samples (cfu/g). The mean count for total viable count ranged between 1.2 x 10^5 and 4.2 x 10^5 cfu/g, for total staphylococcus count between 1.2 x 10^4 and 6.4 x 10^4 cfu/g, for coliform count between 1.0 x 10^4 and 3.0 x 10^4cfu/g, total listeria count recorded no growth on the plates and total campylobacter incident in sample 2 and sample 9 with 1.0x10^4cfu/g and 1.3x10^4cfu/g respectively. The control (laboratory) sample had the least total plate counts with zero coliform growth and fell within satisfactory microbiological quality. Food standards, declares ready to eat foods with aerobic plate counts <10^4 as satisfactory. This may be due to better hygiene rules observed during processing. However, the level of microbial contamination could be associated with inadequate handling and processing by processors and vendors, contamination caused by storage facilities, either poor hygiene or poor quality of water used. Similarly, the extensive mixing during processing could have introduced contaminants via food handlers, cooking utensils and from the environment.

The presence of coliform in the street vended "eggroll" samples is undesirable and suggests possible faecal contamination. The presence of *Staphylococcus aureus* is largely as a result of human contact and this suggest poor hygiene practices of the vendors since the organism is a normal flora of the skin and nasal passage (Nichols et al., 1999). Five genera of bacteria were isolated from the egg roll samples. The isolates were identified as *S. aureus, E. coli, klebsiella spp, Campylobacter spp, Salmonella typhi* by comparing their morphological and biochemical characteristics

(Catalase, Oxidase, Coagulase, Indole, Urease, Sugar tests etc) with standard reference organisms (Olutiola et al., 1991).

Table 2 shows the morphological and biochemical characteristics of bacteria isolates from egg roll samples. According to Bibeki (2001), contamination of food items by specific species of microorganisms is largely due to the presence of these organisms and their entrance into food or beverage as a result of poor hygiene and sanitation.

The isolation of *Klebsiella spp, staphylococcus aureus, E. coli*, and *Salmonella* spp, corroborate the findings of Oranusi *et al*. (2013); Taulo *et al*. (2008) in which these organisms were implicated in ready-to-eat foods.

Table 1: Mean colony forming unit of the total bacterial per gram of egg roll samples (cfu/g).

Tag	Sample	TVC (cfu/g)	TLC (cfu/g	TSC (cfu/g)	TCC (cfu/g)	TC'C (cfu/g)
1	Control	1.4 x 103	NG	1.3 x103	NG	NG
2	Kuto(2)	4.2 x 105	NG	3.3 x104	3.0x104	1.0x104
3	Panseke(3)	2.8 x 105	NG	1.2 x 104	2.5x104	NG
4	Kuto	1.2 x 105	NG	2.0 x 104	1.1 x104	NG
5	Camp	2.4 x 105	NG	3.6 x 104	1.2 x104	NG
6	Kotopo	2.7 x 105	NG	1.2 x 104	2.5 x104	NG
7	Panseke	2.7 x 105	NG	5.4 x 104	1.0 x104	NG
8	Obantoko(1)	2.4 x 105	NG	1.4 x 104	1.2 x104	NG
9	Kuto(3)	3.2 x 105	NG	2.3 x 104	1.2 x104	1.3x104
10	KutoPark(1)	2.2 x 105	NG	3.8 x 104	2.8 x104	NG
11	Olu Omo	1.3 x 105	NG	2.0 x 104	1.1 x104	NG
12	Panseke Park	2.2 x 105	NG	6.4 x 104	1.8 x104	NG
13	Obantoko	1.4 x 105	NG	2.3 x 104	1.0 x104	NG

KEY: NG = No Growth, TVC= Total Viable Count, TLC= Total Listeria Count, TSC= Total Staphylococcus Count TCC= Total Coliform Count, TC'C= Total Campylobacter Count

Table 2: Biochemical and morphological characteristics of bacterial isolate from Eggroll samples

Parameters	Isolates				
	LTNS	LTNE	LTNK	LTNST	LTNC
Most probable organism	SA	EC	KA	ST	CC
Gram staining	+	-	-	-	-
Shape	C	R	R	R	R
Motility test	-	+	-	-	-
Catalase test	+	+	-	+	+
Oxidase test	-	-	+	-	-
Coagulate test	+	-	-	-	-
Urease test	+	-	+	+	+
Indole test	+	+	+	+	+
Methyl red	-	+	+	+	+
Voges Proskauer test	-	-	-	-	-
Gelatin hydrolysis	+	-	+	-	-
Starch hydrolysis	+	+	-	-	+
Casein hydrolysis	-	+	-	+	-
Citrate utilization	+	+	-	-	+
Pigmentation	+	-	+	-	-
H_2S Production	+	-	-	+	-
O_2 Relationship	a	a	a	a	a
Fructose	A	A/G	A	A/G	A
Sucrose	A	A/G	A	A/G	A
Lactose	A	A/G	A	A/G	A
Mannitol	A	A/G	A	A/G	A/G
Arabinose	A	A/G	A	A	A/G
Xylose	A	A/G	A	A	d
Dulcitol	A	A/G	A	A/G	d
Raffinose	A	A/G	A	A/G	A
Glucose	A	A/G	A	A/G	A/G
Maltose	A	A/G	A	d	A/G
Adonitol	A	A/G	A	d	A/G
Sacchrose	A	A/G	A	A	A

SA- *Staphylococcus aureus,* EC- *E. coli,* CC- *Campylobacter coli*, KA-*Klebsiella aerogenes,* ST- *Salmonella typhi*… - (Negative), + (positive). R-Rod, C- Cocci, A- Acid production, A/G-Acid/Gas production, d-Doubtful, a- Aerobic

KEYS:
LTNS-Isolates from sample1(control), sample2 (Kuto2), sample3 (Panseke3), sample4 (Kuto3), sample5 (Camp), sample6(Kotopo), sample8(Obantoko), sample9(Kuto), sample10(Kuto park), sample11(Olu omo), sample12(Panseke park)

LTNE- Isolates from sample2 (Kuto2), sample5(Camp), sample6(Kotopo), sample7 (Panseke), sample8 (Obantoko1), sample13 (Obantoko)

LTNK- Isolates from sample2(Kuto2), sample3(Panseke), sample4(Kuto3), sample7 (Panseke), sample9 (Kuto), sample10 (Kuto park), sample12 (Panseke park), sample13 (Obantoko)

LTNST- Isolates from sample3 (Panseke3), sample4 (Kuto3), sample6 (Kotopo), sample9 (Kuto), sample11(Olu omo).

LTNC- Isolates from sample2 (Kuto2), sample9 (Kuto)

Table 3 shows the level of heavy metal contamination in eggroll sample from the three processors, the nine vendors in the study areas and the egg roll produced in the laboratory (control). Lead was not detected in any of the samples from the processing and vending centers and in the laboratory. The level of chromium ranged between 3.50 mg/g and 12.50 mg/g and the level of cadmium ranged between 0.750 mg/g and 3.500 mg/g. Cadmium and chromium contamination of Egg roll in these locations may be connected to their sources of water supply since many of the processors of the eggroll live in rural areas where well water mostly serve as the source of water for cooking or cottage level processing of food (Atolagbe, 2010). Accumulation of heavy metals can cause irreversible damages to the body, hence not only food borne pathogens are of major concern to people everywhere but also food exposure to pesticides, chemicals and heavy metals is a concern all over the world. Findings revealed that street vended Egg rolls sold within Abeokuta metropolis were contaminated with various microorganisms, and have microbial loads within unsatisfactory microbiological quality (ICMSF, 1974). Food standards, declares ready to eat foods with aerobic plate counts $\geq 10^5$ as unsatisfactory.

Table 3. Heavy metal composition of Egg roll samples

Tag	Sample	Chromium(Cr) (mg/g)	Cadmium(Cd) (mg/g)	Lead(Pb) (mg/g)
1	Control	2.10±0.14^{a}	0.35±0.21^{a}	0±0^{a}
2	Kuto2	9.00±0.71^{ef}	2.25±0.35^{cd}	0±0^{a}
3	Panseke3	11.25±0.35^{hi}	2.00±0.71^{cd}	0±0^{a}
4	Kuto3	9.50±0.71^{fg}	2.00±0.71^{cd}	0±0^{a}
5	Camp	4.50±0.71^{bc}	3.50±0.00^{e}	0±0^{a}
6	Kotopo	12.50±0.71^{i}	1.25±0.35^{bc}	0±0^{a}
7	Panseke	6.00±0.71^{cd}	1.75±0.35^{bcd}	0±0^{a}
8	Obantoko1	4.25±1.10^{b}	2.75±0.35^{de}	0±0$_{a}$
9	Kuto	7.50±0.71^{de}	1.25±0.35^{bc}	0±0^{a}
10	Kuto park1	3.50±0.71^{b}	1.25±0.35^{bc}	0±0^{a}
11	Olu omo	9.50±0.71^{fg}	2.50±0.71^{d}	0±0^{a}
12	Panseke Park	7.50±0.71^{de}	0.75±0.35^{ab}	0±0^{a}
13	Obantoko	10.75±1.10^{gh}	1.75±0.35^{bcd}	0±0^{a}

Mean values having different superscripts show significant differences (P< 0.05) across the column

CONCLUSION

Findings revealed that street vended Egg rolls sold within Abeokuta metropolis were contaminated with various microorganisms, and have microbial loads within unsatisfactory microbiological quality. The heavy metals result showed contamination of all samples with cadmium and chromium and absence of lead in all samples and which may be connected to their sources of water supply.

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