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Original Article

Nutritional and Microbial Profile of a Traditional Food Condiment

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Abstract

This study evaluated the nutritional and microbial profile of a traditional condiment ('yagie') used as a rub for skewered meats popularly known as 'khebab' in Ghana. The analyses included proximate composition, total bacteria count (TBC) and identification of pathogens. Bacteria were Gram stained in order to differentiate them. Moisture, fat, crude fibre, crude protein and ash contents of the samples ranged from 8.67% to 18.17%, 14.17% to 25.67%, 1.37% to 9.60%, 28.37% to 35.07% and 3.00% to 20.17% respectively. Total bacteria counts ranged from10^2 to 10^6cfu/g, with 3.5 $\times 10^6$ cfu/g being the highest (C2KVT) recorded in 'yagie'. The mean bacterial count was 4.3 $\times$ 10^5 cfu/g. Microorganisms identified were *Salmonella*, *Escherichia coli*, *Clostridium perfringens* and *Bacillus species. Salmonella* was present in 65%, *Bacillus species* in 95**%**, *Clostridium perfringens* in 50% and *E. coli* in 50% of the samples analyzed. Also 95% of the organisms were Gram-positive spore formers while 5% were Gram-positive cocci. These findings suggest that though 'yagie' could be a good source of crude fat, fibre and protein, it could contain pathogenic organisms which can potentially contribute to food poisoning among consumers.

Keywords: 'Yagie', microbial profile, proximate composition, condiment, 'khebab'.

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Introduction

Food condiments have been defined by the International Organization for Standardization (ISO, 1972) as vegetable products or mixtures, free from extraneous matter, which are used for flavoring, seasoning or imparting aroma in foods. They are normally not eaten as a principal food during a meal but are mostly added to other foods to improve or alter the flavor (flavor enhancers) in many traditional dishes including; sauces and soups (Azokpota *et al.,* 2011). Condiments can also act as texture enhancers; and in some cultures as a complement to a main dish (Redmond, 2007). There are usually two types of condiments, namely simple and compound condiments. Common examples of simple condiments are celery salt, garlic salt, pepper salt and onion salt, while compound condiments include chili sauce, sour sauce, soya sauce, prepared mustard and mint sauce (Ravindran *et al.,* 2002).

Examples of some condiments produced in different nations are as follows: 'netetu' in Senegal (N'dir *et al.,* 1994), 'iru' or 'dawadawa' in Nigeria and Ghana (Odunfa, 1981; Shao, 2002), 'soumbala' in Burkina Faso (Diaware *et al.,* 1998), 'afitin' and 'sonru' in Benin (Azokpota *et al.,* 2011), 'natto' in Japan, 'kinema' in Nepal (Wang and Fung, 1996) and 'yagie' in Nigeria and Ghana (Adebesin *et al.,* 2001).

Due to their nutritional contents, condiments are mostly used as substrates (non-meat proteins) and also as functional ingredients in fabricated foods. Achi (1991) suggested that the inclusion of condiments could break the monotony of such supplementations and bring about variety in foods because most traditional diets in the western part of Africa lack variety and are mostly consisting of staple foods, with supplements of beans, rice and cocoyam, and their use usually depends on availability and seasonality. 'Yagie' is a very common condiment among the Hausa people of Ghana and Nigeria and is said to have originated from northern Nigeria. It has been used for a long time as a condiment for 'khebab'. It consists of many different mixtures of spices and additives. According to Nwaopara *et al.,* (2004) and Adebesin *et al.,* (2001), some of the constituent ingredients of 'yagie' are: African Negro pepper, groundnut flour ('kulikuli'), ginger, powdered pepper, common salt and Maggie cubes. Spices and condiments, like any other food substance in this world are also not free from microbial association. Spices may acquire microorganisms during growth and developmental stages or even during later stages when they are collected, during processing, storage and/ or marketing (Mahbubar *et al.,* 2012). Although the spices used in the preparation of 'yagie' may have some antimicrobial properties, some bacteria and fungi can utilize these spices for their growth (Beales, 2002).

This study was therefore undertaken to evaluate the nutrient composition and microbial profile of the condiment 'yagie'. Specifically the protein, fat, ash, fiber and moisture contents of 'yagie' were determined. The microbial load of 'yagie' was assessed and specific harmful microbes present were isolated and identified.

Materials and Methods

Study Area and Data Collection

The study was undertaken in the Kumasi metropolis of the Ashanti region in Ghana. Samples were obtained from 'yagie' producers and some 'khebab' vendors in the metropolis in order to obtain quantitative data. Samples of 'yagie' obtained from these respondents were immediately transported in chest freezers to the laboratory for analysis.

The samples were collected from six different markets namely; Kumasi central market, Asafo market, Mayanka market, Aboabo station market, Akwatia line market, French line market and the Ayigya market, and also from five randomly selected khebab vendors. The samples from each location were collected in sterile sampling bags, identified with codes and treated separately. In order not to reveal the identity of 'yagie' producers and 'khebab' vendors visited during this study, all samples collected were purposefully coded as shown in Table 1.

Table 1: Sample identity.

Sample No.	Code
1	AMA
2	BMM
3	C1KVT
4	C2KVT
5	DKVM
6	EMAS
7	FMC
8	GMAL
9	HMFL
10	IMA
11	KKVQH
12	LKVIH
13	MMC
14	NMC
15	OMA
16	PMA
17	QMA
18	RMM
19	SMM
20	TMM

Laboratory Analysis

Proximate Analysis

The proximate compositions (moisture, crude protein, crude fat, crude fibre and ash) of the samples were determined using methods recommended by the Association of Official Analytical Chemists (AOAC, 2002). All determinations were made in triplicate.

Microbial Study and Identification

Total Bacteria Count

The bacterial load of the samples was determined by transferring one gram of sample into 9ml of 0.1% peptone water. A vortex mixer was used to shake the tubes and serial dilutions of up to $1:10^6$ were made. 1ml aliquot were obtained from each of the dilutions and plated on 20ml nutrient agar. The plates were incubated at 35°C±2 for 24 hours. After the incubation time, colonies formed were counted using a colony counter (STUART® Scientific, UK). The counts of bacteria were expressed as colony forming units per gram of sample (cfu/g) (Chaudhary and Padma, 2014).

Gram Staining

The Gram staining procedure was carried out according to the method described by (Cheesebrough, 2000). Bacteria smears were stained with crystal violet and allowed to set for 60 seconds. Each was then rinsed with slow running distilled water, drained and stained again with Gram's iodine for 60 seconds. The slide was washed with distilled water and decolorized in 95% ethyl alcohol. Lastly, each smear was again stained with safranin for 60 seconds after which they were washed and drained. The slides were dried and examined under the light microscope using an oil immersion lens of ×100 magnification after a small drop of immersion oil was placed directly onto the smear.

Detection of E. coli

Detection of *E. coli* was carried out according to the method described by (Washington *et al.*, 2006). One milliliter (1ml) of inoculum was transferred onto sterile petri dishes. This was followed by aseptically pouring 20 ml of molten MacConkey agar. The inoculated medium was mixed by swirling the plate, after which it was allowed to solidify. The plates were incubated at

37°C for 24 hours. After incubation lactose-fermenting organisms grew as pink colonies and the non-lactose fermenters grew as clear colonies. Biochemical tests were done to confirm *E. coli* using the indole and citrate tests (Cheesebrough, 2000).

Detection of Clostridium Perfringens

This was determined according to the method described by Cheesebrough (2000) using neomycin blood agar. Streaks of homogenate were made on the agar and anaerobically incubated for 24 hours at 37°C. After incubation, the plates were observed for large β-haemolytic colonies.

Detection of Salmonella

Salmonella detection was done according to the method described by Cheesebrough (2000). One millilitre (1ml) of inoculum from dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} were transferred into petri dishes containing bismuth sulphite agar and labeled accordingly.

Afterwards they were incubated for 48 hours and checked for black colonies with a surrounding metallic sheen from hydrogen sulphide production and reduction of sulphite to black ferric sulphide.

Results and Discussion

Proximate Composition

The results obtained for the percentage ash, crude protein, crude fat, crude fibre and moisture contents of 'yagie' from the Kumasi metropolis of Ghana are shown in Table 2.

Table 2: Proximate composition of 'yagie' in the Kumasi metropolis.

Sample code	Proximate compositions (%)				crude fibre
	Moisture	crude protein	crude fat	ash	
AMA	11.33^{i}	35.07^{abc}	20.17^{de}	9.00^{f}	2.11^{q}
BMM	14.33^{e}	34.80^{de}	23.50^{b}	7.50^{h}	2.94^{p}
C1KVT	11.17^{ij}	28.37^{g}	18.00^{g}	20.17^{a}	1.37^{r}
C2KVT	18.17^{a}	31.33^{defg}	21.17^{cd}	9.00^{f}	4.57^{j}
DKVM	14.17^{e}	33.27^{abcd}	23.33^{b}	7.67^{h}	4.00^{m}
EMAS	12.33^{fg}	33.17^{abcde}	25.67^{a}	10.17^{e}	3.28^{o}
FMC	16.33^{c}	32.40^{bcde}	21.50^{c}	8.50^{g}	5.66^{g}
GMAL	15.50^{d}	32.57^{bcde}	21.16^{cd}	7.33^{h}	3.29^{o}
HMFL	15.17^{d}	32.67^{bcde}	19.33^{ef}	6.50^{i}	3.49^{n}
IMA	8.67^{l}	29.80^{efg}	20.33^{de}	10.17^{e}	5.35^{i}
KKVQH	14.33^{e}	28.13^{g}	14.17^{j}	13.50^{d}	7.36^{b}
LKVIH	10.67^{jk}	31.80^{cdef}	18.67^{fg}	14.33^{c}	6.79^{c}
MMC	18.33^{a}	36.17^{a}	21.00^{cd}	8.50^{g}	5.60^{h}
NMC	17.16^{b}	33.93^{abcd}	21.50^{c}	3.17^{k}	6.40^{e}
OMA	11.17^{ij}	33.70^{abcd}	20.17^{de}	3.00^{k}	6.69^{d}
PMA	12.00^{gh}	28.73^{fg}	14.67^{j}	15.67^{b}	4.24^{l}
QMA	12.67^{f}	33.87^{abcd}	14.33^{j}	13.50^{d}	9.60^{a}
RMM	12.33^{fg}	36.33^{a}	15.67^{i}	7.33^{h}	5.71^{f}
SMM	11.50^{hi}	36.27^{a}	16.67^{h}	5.67^{j}	4.37^{k}
TMM	10.17^{k}	35.43^{ab}	16.50^{hi}	8.50^{g}	5.63^{g}
Sem	0.349	0.378	0.248	0.533	0.248

^{abcdefghijklmnopqr}: Means in the same column with different superscripts are significantly different ($p<0.05$).

Moisture

The moisture content of the samples ranged between 8.67% (IMA) and 18.33% (MMC). Though MMC had the highest moisture content, the differences observed between MMC and C2KVT (18.17%) were not statistically significant ($p>0.05$). IMA seemed to have lower moisture contents, and was significantly ($p<0.05$) different from the moisture contents of samples from all the other locations. The differences in the moisture contents of the samples were most probably due to the differences in the ingredients used, because whereas some 'yagie' producers used dried spices others were using fresh spices in different proportions. 'Yagie' sample containing lower moisture contents are more likely to have longer shelf compared to those with higher moisture contents.

Crude Protein

The percentage crude protein contents of the samples ranged from 28.13% (KKVQH) to 36.33% (RMM). RMM had the highest protein content, but this was not significantly different ($p>0.05$) from SMM, TMM, QMA, OMA, NMC, MMC, EMAS, DKVM, BMM and AMA. The observed lower protein content of KKVQH were however not significantly different ($p>0.05$) from the protein contents of C1KVT, C2KVT, IMA and PMA. Again, these differences in the crude protein contents were probably due to type of ingredients used by the different producers of 'yagie'. It was observed that though some 'yagie' producers used similar ingredients in processing, the specific proportions in the overall product formulations varied from one processor to the other.

Fat

Fat content ranged from 14.17% (KKVQH) to 25.67% (EMAS). EMAS, with the highest crude fat content was significantly different ($p<0.05$) from all the other samples while KKVQH had the lowest fat content, which was not significantly different ($p<0.05$) from PMA and QMA. The differences and variability observed in fat contents of the samples is most probably due to components like the groundnut ('kulikuli')-which is reported to contain high fat contents (Ranken *et al.,* 1997; Eshun *et al.,* 2013). High fat contents, according to Saltzman (1997) could increase the energy level of the product. However, such samples are more likely to be prone to oxidative rancidity which could affect their flavor when used on meats.

Ash

The observed ash contents of 'yagie' were between 3.00% (OMA) and 20.17% (C1KVT). Though C1KVT seemed to be the highest in ash content, the observed differences were not statistically significant among all the other samples. OMA had the lowest ash content which was not significantly different ($p>0.05$) from samples from NMC. The observed differences in the ash contents are most probably due to their levels of contamination with sand and other foreign materials. During sample collection it was observed that some 'yagie' producers placed their equipment and ingredients on the bare ground during preparation, so it is possible that such practices contributed to contaminate the products with sand.

Crude Fibre

The crude fibre content of the samples ranged from 1.37% (C1KVT) to 9.60% (QMA). It was revealed that QMA had the highest crude fibre content which was significantly different ($p<0.05$) from all the other samples. The differences and increased variability observed in the crude fibre contents (1.37% to 9.60 %) were attributed to the different ingredients used in the preparation of 'yagie'. Crude fibre contents of up to 4.1% (ginger), 5.55% (groundnut) and 2.61% (pepper) have been reported by Dei-tutu and Risch (1976), Eshun *et al.,* (2012) and Otunola *et al., (*2010) respectively. The presence of fibre in our diets is necessary for maintaining bowel health, normalizing bowel movement and helping in the prevention of some chronic diseases including colon cancer, cardiovascular diseases and several other disorders (Williams, 1995; Slavin, 1987; Johnson & Southgate, 1994).

Microbiological Analysis

Total Bacteria Count (TBC)

The results obtained after culturing bacteria for 24 hours are shown in Table 3. The mean bacteria count of the samples was 4.3×10^5cfu/g. It was

observed that C2KVT had the highest bacteria load. This observation is most probably due to sub-standard production practices used by those involved in its preparation. As stated earlier, it was observed that some 'yagie' producers placed their ingredients on the bare ground during preparation while others did not wash the ingredients before use. Also some processors did not wash the equipment used before or after 'yagie' preparation. More so, some producers did packaging of their products on the bare ground; a practice that could compromise the microbial status of a food item. All these occurred because there were no laid down standards for the preparation of 'yagie' in Ghana.

Table 3: Total bacteria counts (TBC) after culturing for 24 hours.

Sample code	TBC* (cfu/g)
AMA	1.4×10^5
BMM	2.8×10^5
C1KVT	1.3×10^6
C2KVT	3.5×10^6
DKVM	7.3×10^5
EMAS	5.7×10^5
FMC	2.3×10^5
GMAL	1.7×10^5
HMFL	1.4×10^5
IMA	3.0×10^5
KKVQH	5.5×10^2
LKVIH	6.7×10^4
MMC	3.7×10^5
NMC	6.6×10^4
OMA	1.8×10^4
PMA	4.0×10^5
QMA	1.1×10^4
RMM	1.2×10^4
SMM	5.5×10^2
TMM	2.4×10^5

*Average of triplicates. The mean bacterial count = 4.3×10^5 (cfu/g).

KKVQH and SMM had the lowest loads of bacteria. This observation may be attributed to better manufacturing practices observed by these producers and also most probably, due to anti-microbial properties of the ingredients they used (Mahbubar, 2012). C1KVT and C2KVT had high bacteria counts per the criteria of the Food and Administration Manual (FAM) (1995).

Bacteria Identification

After comparing their morphological and biochemical characteristics, bacteria isolates were provisionally identified. All the isolates identified in this study were gram positive spore-forming rods with the exception of those obtained from EMAS, which were gram positive non-spore formers. It was observed that 95% of bacteria isolates belonged to the genus *Bacillus* and were spore-forming, while 5% were c*occi* (Table 4). *E. coli* was detected in 50% of the samples, while *salmonella* was detected in 65% and C*lostridium perfringens* in 50% of 'yagie' sampled. The presence of these organisms in 'yagie' is an indication of fecal contamination. Adequate and urgent reforms need to be deployed immediately in order to protect consumers because these organisms are known to cause food borne illnesses and poisoning (Sousa, 2006).

The findings of this study suggest that the sole aim of adding 'yagie' to meat is to improve sensory attributes; but its use has the potential to improve nutritional benefits because it is high in protein and fat. The microbial loads of the 'yagie' used in this study were found to be generally acceptable (FAM,

1995), however there were indicator organisms, which under favorable conditions may multiply to cause spoilage of the product as well as render it as vehicle for food infection and poisoning to potential consumers. Hence the Food and Drugs Authority in Ghana should take immediate steps to ensure consumer safety from 'yagie' consumption in Kumasi. 'Yagie' should be produced under strict hygienic conditions with a primary focus on consumer safety and product quality. It is recommended that further studies should be conducted in order to obtain a standard procedure for producing 'yagie' in Ghana.

Table 4: Microbiological analysis: Types of microbes detected in 'yagie'.

Sample code	Gram stain	*E. coli*	*Salmonella*	*Clostridium perfringens*
AMA	Gram positive bacilli	-	-	+
BMM	Gram positive bacilli	+	+	+
C1KVT	Gram positive bacilli	+	+	-
C2KVT	Gram positive bacilli	-	+	+
DKVM	Gram positive bacilli	+	+	-
EMAS	Gram positive cocci	+	+	-
FMC	Gram positive bacilli	-	+	+
GMAL	Gram positive bacilli	+	+	+
HMFL	Gram positive bacilli	+	+	+
IMA	Gram positive bacilli	+	+	+
KKVQH	Gram positive bacilli	-	-	-
LKVIH	Gram positive bacilli	-	-	+
MMC	Gram positive bacilli	+	+	-
NMC	Gram positive bacilli	-	+	-
OMA	Gram positive bacilli	+	-	+
PMA	Gram positive bacilli	-	-	-
QMA	Gram positive bacilli	-	-	-
RMM	Gram positive bacilli	+	+	+
SMM	Gram positive bacilli	-	-	-
TMM	Gram positive bacilli	-	+	-

Key: + = Detected, - = Not detected.

Statistical Analysis

Data collected from the proximate determination were analyzed using the Statistical Package for Social Science (SPSS) (2007) version 16.0 and Duncan's test of homogeneity was used to determine significant differences between treatment means at 5%.

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