Proximate Composition and Microbiological Analysis of *Yaji* (Spiced Pepper Mixture) Sold Within Kano Metropolis, Nigeria

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ABSTRACT: Food safety must be given high priority in any society. This study was aimed at assessing the proximate composition and microbiological quality of *yaji*. A total of nine different *yaji* were randomly purchased from retail stores within Kano metropolis while three samples were prepared in the laboratory as control. The result of the study revealed that the *yaji* samples studied had moisture content ranging from5.00-10.70%, ash content of 17.42-26.30%,crude protein content of 17.50-32.43%, fat content of 1.89-4.05%, and carbohydrate content of 36.69-47.60%.The microbiological analysis showed that the purchased yaji samples were contaminated with aerobic mesophilic bacteria and fungi isolates.Aerobic mesophilic bacterial count, fungal count and coliform count ranged between 3.35×10^6 - $>3.00\times10^7$cfu/g,$<1.00\times10^3$ – 9.60×10^6cfu/g, and 3.6 ->1100MPN/g respectively. *Staphylococcus aureus, Escherichia coli, Salmonella, Enterobacter*, and *Klebsiella species* were isolated. *S.aureus* was the predominant bacteria isolated with frequency of occurrence of 34.35%. The fungi isolated were *Fusarium, Rhizopus* and *Aspergillus* species. The predominant fungal isolate was *Aspergillus* spp with frequency of occurrence of 60%.This study showed that yaji samples though nutritionally rich were found to be contaminated with pathogenic microorganisms and had higher microbial counts compared to the maximum acceptable levels provided by the ISO and FAO, thus posing serious health hazards to consumers. Standard hygienic measures during preparation of yaji are highly recommended in order to reduce microbial load to acceptable limit.
Key words: *Yaji* (spice pepper), proximate composition, microbiological analysis.

INTRODUCTION

Powdered spiced pepper sauce popularly known as *yaji* in the northern part of Nigeria was named after a 14th century Hausa ruler called "*Yaji* (meaning the 'hot one')" (Afrolems, 2012). The principle component of *yaji* is chili pepper to which other ingredients/spices such as table salt, ginger, cloves and black pepper are added. This type of mixture is commonly referred to as the red chili or red pepper or *Jan yaji* in Hausa. Currently, other food seasonings added to *yaji* includes garlic, seasoning cubes (*maggi*) and monosodium glutamate, additionally, locust beans-based *yaji* commonly referred to as *yajin daddawa* in Hausa contains locust beans powder in substantial quantity as an important ingredient in the *yaji*. Other forms of yaji may include garlic-based *yaji* called *yajin tafarnuwa* and groundnut cake powder *yaji (yajin kuli-kuli).* Previous studies by Nwaopara *et al.,* (2004) revealed thatthe spices in yaji are ginger, garlic, cloves, red pepper, and black pepper.

Akinsola (2015) reiterated that the spice blend used in making *yaji* provides an easy and delicious way of using ingredients to provide a rich source of vitamins, minerals, proteins, fibers and antioxidants. Akinsola(2015) further noted that most of the ingredients used in making *yaji* combats oxidation stress, fights inflammation and also helps to reduce the risk of chronic diseases. Example ground nut powder acts as an antioxidants and anti-inflammatory (Akinsola, 2015).

Measurement of proximate profile of *yaji* is often necessary to ensure that they meet the requirement of food regulations and commercial specification (Waterman, 2000).Proximate analysis is the partitioning of compounds in food into six major categories based on the chemical properties of the compounds. The six categories include moisture, ash, crude proteins, crude lipid, crude fiber, nitrogen-free extracts or digestible carbohydrates (Maurice, 2010). One of the most major challenges that interfere with studies relating to *yaji* as identified by Oko *et al.* (2015) is the absence of labels in *yaji* containers which will indicate vital information such as name and address of producers, nutritional content and recommendations for storage and best before date for human consumption.

Spiced pepper (*yaji*) may be contaminated with microorganisms because of the condition under which the individual ingredients were cultivated, harvested and processed (Emmanuel-Ikpeme *et al.,* 2014). Contaminated spices have been reported to have been the cause of certain food-.borne illnesses and spoilage (Ahene *et al*., 2011). Indigenous micro flora of plant, air, dust, using contaminated water, animal and human excreta, pre- and postharvest procedures including processing, storage, and distribution may be the sources of microbial contamination of spiced pepper (Ahene *et al*., 2011). As such posing health problems on consumers who often add spiced pepper to their food without further processing (Emmanuel-Ikpeme*et al*., 2014). Previous studies have demonstrated that spiced pepper could be contaminated by total heterotrophs, *Bacillus cereus, Clostridium perfringens, Escherichia coli, Salmonella species, Shigella species, Pseudomonas aeruginosa, Aeromonas species, Staphylococcus aureus* and toxigenic molds (Aksu *et al*., 2000; Cadlish *et al*., 2001; Banarjee and Sarkor, 2003; Kontor, 2015). The presence of contaminating microorganisms in a product as *yaji* which is meant for consumption and is being patronized by a wide range of people of different social and economic class becomes public health concernissue. Therefore this study was aimed at determining the proximate composition and microbiological analysis of *yaji.*

MATERIALS AND METHODS

Source of samples

A total of 9 random *yaji* samplesof three different kind (garlic-based, locust beans-based and red chili) were purchased from retail shops in Rimi market within Kano metropolis. All the samples basically contain chilly, cloves, West African blackpepper, salt and seasoning cubes. The garlic-based *yaji* additionally contains garlic in substantial quantity and the locus beans-based *yaji* also contains locust beans in substantial quantity. The samples were immediately taken to the Laboratory of Microbiology, Bayero University Kano for further analysis.

Preparation of different combinations of *yaji* to serve as control

Garlic-based, locust beans-based and chili*yaji* were prepared in the laboratory following the method adopted by Ugbogu *et al.*(2018) with slight modifications. The *yaji* spices were sorted and washed with sterilized water and then dried in the hot air oven set at 60°C for 72hours and weighed before grinding. These spices include garlic (250g), ginger (20g), black pepper (25g), red pepper (300g) cloves (25g), locust beans (50g), seasoning cubes (200g) and table salt (50g). All the spices excluding garlic, and locust beans were subsequently mixed together and ground into powder using a properly washed wooden mortar and pestle. The spices were sieved and divided into three parts. Powdered garlic was added to the first portion to form garlic-based *yaji* (*yajin tafarnuwar*) coded as Y_{10}, locust beans was added to the second portion to form locust beans-based *yaji* (*yajin daddawa*) coded as Y_{11}, while the third portion without any addition was left as red chili (*Janyaji*) coded as Y_{12}.The*yaji* combinations were then packed in three sterile sample containers for further analysis.

Sample preparations and serial dilution

Sample preparations were carried out according to the method described by FAO (1979). Twenty five (25g) grams of each of the nine samples and control wasweighed and homogenized separately in 225ml of peptone water using an electronic blender. These were labeled as 1:10 dilution and further diluted to $1:10^7$.

Total Aerobic plate count

This was carried out according to the method of Shamsuddeen (2009). One milliliter (1ml) of inoculum from 10^{-3}, 10^4 and, 10^{-5}dilutions prepared from above weretransferred into duplicate Petri dishes which werelabeled accordingly. This was followed by pouring aseptically 20ml of molten nutrient agar. The inoculated plates were mixed by swirling the plates and this was later allowed to solidify. The plates were then incubated at 37°C for 24hrs.

After incubation, plates containing 30-300 colonies were selected and the colonies counted and recorded.The average was taken and the number obtained wasmultiplied by the inverse of the dilution factor. This gavethe number of colony forming units per gram of each sample (*cfu/g*).

Enumeration and Detection of Coliforms

This was carried out according to the method described by Atlas (1997). In this method, a set up consisting of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tube were autoclaved to sterilize and expel air. Inoculation were made from the serially diluted samples as follows: From the 1:10 dilution, 1ml of inoculum was transferred to each of the first three of the 9 test tubes containing 9ml of lactose broth to make 1:100 dilution. Then 1ml also was transferred from 1:100 dilution to each of the second set of three test tubes of lactose broth (to get 1:1000 dilution) and finally 1ml of inoculum was transferred from 1:1000 dilution to each of the last three tubes, to make 1:10000 dilution. All the 9 test tubes were incubated at 37ºC for 24 hours and another 24 hours in the absence of gas (presumptive test).

Following 24 hours of incubation the tubes were observed for gas production and the number of gas positive tubes were compared with the most probable number (MPN) table to estimate the most probable number of coliforms per gram of sample. Presence of *E.coli* was confirmed by transferring a loopful of inoculum from gas positive tubes into tubes containing 9ml of Brilliant Green Lactose Broth (BGLB) and were incubated at 37^{o}C for 24 hours.

Following 24 hours of incubation the tubes were observed for gas production and a loopful of broth from gas positive tubes were streaked onto Eosin methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Following incubation, colonies which formed bluish black colour with green metallic sheen, and reddish colonies were noted and isolated on agar slants. This is called the confirmatory test. Also colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 37ºC. The tubes were observed after 24 hours for gas production to confirm fecal coliform. The presence of *E. coli* was confirmed on the basis of Indole production by using tryptone water and Kovac's reagent (Shamsuddeen, 2009).

Fungal count

Total fungal count were determined by pour plating on potato dextrose Agar plates and incubated at room temperature (26°C) for 72 hours. Pure cultures of fungal isolates were obtained on potato dextrose agar plates. Smear of the pure culture of the fungal isolate were made on clean glass slides and lactophenol cotton blue reagent was added for 1 minutes. It was then rinsed with distilled water and vied under the microscope (Carlson and Ensley, 2003).

Proximate configuration

The proximate analysis were carried out according to standard method of the Association of Official Analysis of Chemist (AOAC) and those of Ngozi *et al*., 2017.

Determination of moisture content

The moisture content was determined by the method described by AOAC (2005). In this process, 2g of the sample was dried in a hot air oven for 24 hours at 100°C.

The loss in weight was determined and recorded as the moisture content and expressed as;

$$\%\text{moisture} = \frac{w_1 - w_2}{w_1}$$

Where; W_1 = Initial weight of the sample

W_2 = Weight of the dried sample

Determination of ash content

The ash content was determined by dry ashing method as contained in AOAC (2005). In this method, 2g of each of the samples was measured into a porcelain crucible of known weight, the samples was burnt to ash in a muffle furnace for 12 hours at 550°C, it was then transferred to a desiccator where it was allowed to cool and the weight of the ash was finally determined prior to weighing.

The % ash content was calculated as;

$$\%Ash = \frac{w_1 - w_2 \times 100}{w_1}$$

Where W_1 = Initial weight of the sample

W_2 = Weight of the sample after ashing

Determination of protein content

The macro Kjeldahl method (developed by Johann Kjeldahl in 1883) as described by AOAC (2005) was used to determine the crude protein content. 2g of the samples was introduced into a digestion flask. 10g of copper sulphate and sodium sulphate in the ratio of 5:1 and 25ml of concentrated sulphuric acid was added to the digestion flask. The flask was placed into the digestion block in a fume cupboard and heated until frothing ceases giving a clear and light blue colouration. The mixture was allowed to cool and it was diluted with distilled water until it reaches 25ml of volumetric flask.

10ml of the mixture was poured into the distillation apparatus and 10ml of 40% sodium hydroxide was added. The release of ammonia by boric acid was allowed to continue until 10ml of boric acid is treated with 0.02ml of hydrochloric acid and until the green colour changes to purple.

$$\text{Sample (Nitrogen)} + H_2SO_{4(aq)} \rightarrow (NH_4)_2SO_{4(aq)}$$

$$(NH_4)_2SO_{4(aq)} + 2NaOH_{(aq)} \rightarrow 2NH_{3(aq)} + 2H_2O + Na_2SO_{4(aq)}$$

$$NH_{3(aq)} + H_3BO_{3(aq)} \rightarrow NH^+_{4(aq)} + H_2BO^-_{3(aq)}$$

$$H^+_{(aq)} + H_2BO^-_{3(aq)} \rightarrow H_3BO_{3(aq)}$$

The percentage protein content in the sample was calculated as;

$$\%\text{Crude protein} = \%N \times 6.25$$

The nitrogen content of the sample is given by the formula;

$$\%N = \frac{Tv \times Na \times 0.014 \times V1 \times 100}{G \times V2}$$

Where,

Tv = Titre value of the acid (cm^3)

Na = Concentration or normality of the acid

V1 = volume of distilled water used for distilling the digest

V2 = Volume of aliquot used for distillation

G = Original weight of sample used (g)

Determination of lipid content

The Soxhlet method (Semi continuous solvent extraction method) as described by AOAC (2005) was used to determine the lipid content. Two grams (2g) of the predried sample was weighed to the nearest mg into a predried extraction thimble, with porosity permitting a rapid flow of ethyl ether. The sample in the thimble was covered with glass wool. Predried boiling flask was weighed, anhydrous ether (prepared by washing commercial ethyl ether with three portion of H_2O, adding NaOH, and letting it to stand until most of H_2O is absorbed from the ether) was added to the boiling flask and a small piece of metallic sodium was added. Hydrogen evolution was allowed to cease. The boiling flask, Soxhlet flask, and condenser was assembled and the lipid was extracted in the Soxhlet extractor at the rate of five to six drops per second by condensation for about 4 hour, or for 16 hours at the rate of two or three drops per second by heating solvent in boiling flask. The extracted fat was dried in an air oven at 100°C for 30 min, cooled in a desiccator and weighed.

Fat content was calculated as;

$$\%Fat\ on\ dry\ weight\ basis = \frac{weight\ of\ fat\ in\ sample\ (g) \times 100}{weight\ of\ dried\ sample\ (g)}$$

Determination crude fiber content

The crude fiber was determined using the procedure in AOAC (2005). It was determined using the fraction remaining after digestion with standard sulphuric acid and sodium hydroxide under careful controlled condition.

Using this method, 5g of the sample was weighed and fat was extracted with petroleum ether using Soxhlet extractor, the fiber sample was weighed into 500ml prepared sulphuric acid solution. The mixture was boiled for 30 minutes, refluxed 3 times by boiling water and was allowed to continue for another 30 minutes, refluxed 3 times by boiling water and was extracted and dried in a crucible by moistening with small portion of acetone which was permitted to drain, the sample in the crucible was incinerated at 550°C for 3 hours until all carbonaceous matter was burnt. The crucible containing the ash was cooled in a desiccator and weighed.

The percentage crude fiber was calculated as;

$$\%Crude\ fiber = \frac{w_1 - w_2 \times 100}{w}$$

%

Where; W = Weight of the sample

W_1 = Weight of sample and crucible before ashing

W_2 = Weight of crucible and ash

Determination of carbohydrate content

The content of carbohydrate was determined as;$\mathrm{CHO} = 100 - \%(\mathrm{ash} + \mathrm{protein} + \mathrm{fat} + \mathrm{moisture})$

RESULT

The results of the study are shown in tables 1, 2, 3 and 4 respectively. Table 3 shows the microorganisms isolated from the samples with their percentage frequency of occurrence. Table 3 shows that *Staphylococcus aureus, Escherichia coli, Salmonella, Enterobacter,* and *Klebsiella* were the bacteria isolated from the samples in which *Staphylococcus aureus* had the highest frequency of occurrence (34.35%). The fungi isolated were *Fusarium, Rhizopus* and *Aspergillus* species which had the highest frequency of occurrence (60%) (Table 3).

Table 1: Proximate analysis of some *yaji* sample

Sample	Ash (%)	Moisture (%)	Crude protein (%)	Fat (%)	Carbohydrate (%)
Y2	22.96	10.70	17.97	3.30	45.06
Y3	25.82	5.00	18.20	3.39	47.60
Y4	26.30	8.70	17.50	1.89	45.62
Y7	19.91	8.30	26.13	4.09	41.08
Y8	17.42	10.00	32.43	3.67	42.90
Y9	17.56	8.70	26.46	4.65	36.69

KEY:

Y2 and Y7= Chilly or red pepper *yaji* (*Jan yaji*), Y3 and Y8= Locust beans-based *yaji* (*Yajin daddawa*) A, Y4 and Y9= Garlic-based *yaji* (*Yajin tafarnuwa*) A

Table 2: Total viable counts of bacteria, fungi and coliforms of *yaji*samples

Type	Sample	TAMC (*cfu/g*)	FC (*cfu/g*)	CC (MPN/g)
Locust beans-based (yajin daddawa)	Y1	2.91×10^7	7.40×10^5	20
	Y3	$>3.00\times10^7$	4.70×10^6	1100
	Y8	$>3.00\times10^7$	1.10×10^6	1100
	Y11	3.85×10^6	$<1.00\times10^3$	20
Red chili (*Jan yaji)*	Y2	$>3.00\times10^7$	$<1.00\times10^3$	93
	Y5	2.10×10^7	6.55×10^5	75
	Y7	$>3.00\times10^7$	9.75×10^5	28
	Y12	4.10×10^6	$<1.00\times10^3$	11
Garlic-based (*yajin tafarnuwa*)	Y4	$>3.00\times10^7$	9.60×10^6	3.6
	Y6	1.55×10^7	$<1.00\times10^3$	150
	Y9	$>3.00\times10^7$	1.39×10^6	>1100
	Y10	3.35×10^6	$<1.00\times10^3$	3.6

Key: TAMC= Total Aerobic Mesophilic Count, FC= Fungal Count, CC= Coliform Count, *cfu/g*= coliform forming unit per gram, MPN/g= Most Probable Number/gram
Y1, Y3, Y8, and Y11= Locust beans-based yaji (*yajin daddawa*), Y2, Y7, Y5 and Y12= Chili or red pepper yaji (*Jan yaji*), Y4, Y9, Y6 and Y10= Garlic-based yaji (*yaji tafarnuwa*)

Table 3: Percentage frequency of the bacteria isolated from *yaji* samples

S/N	Bacteria Isolated	Frequency	%Frequency
1	*Staphylococcus aureus*	11	34.38
2	*Escherichia coli*	10	31.25
3	*Klebsiella* specie	5	15.25
4	*Enterobacter* specie	3	9.38
5	*Salmonella* specie	3	9.38

Table 4: Percentage frequency of the fungi isolated from *yaji* samples

S/N	Fungi Isolated	(x) Frequency	%Frequency
1	*Fusarium* specie	3	15.00
2	*Rhizopus* specie	5	25.00
3	*Aspergillus* specie	12	60.00

DICUSSION

The findings of the study based on proximate analysis showed that *yaji* samples are rich in carbohydrate, proteins and lipid which are essential to body mass development in humans. The ash contents, crude protein contents and carbohydrate contents of three of the purchased samples were slightly higher than those of the control samples, this may be due to the fact that most of the ingredients used in preparing the *yaji* samples were not measured into appropriate proportions unlike the ingredients used in preparing the control. The moisture and fat contents of all the samples showed very little variation. However, the results of the proximate analysis recorded were slightly lower to that of Joseph and Jeremiah (2015), who recorded 55.15% for carbohydrate, 12.65% for moisture, 12.60% for protein, 15.40% for lipid and 3.4% for ash. The proximate analysis also indicated that *yaji* could be stored for a long period due to its low moisture content.

The results of the Microbiological analysis of *yaji* samples shows high aerobic plate count and fungal counts. Some of the values obtained exceeded the maximum acceptable limit stated by the Food and Agricultural Organization FAO (1979), according to which the aerobic mesophilic count and fungal should not be greater than 10^6 and 10^4*cfu/g* respectively.

The aerobic bacterial count, fungal count, and coliform count ranged from 3.35×10^6 - $>3.00\times10^7$*cfu/g*, $<1.00\times10^3$ – 9.60×10^6*cfu/g*, and 3.6 - >1100*MPN/g* respectively. This is inline with the work of Shamsudeen, (2009) whose bacterial and fungal counts on the spices used in producing *kilishi* (a local dried meat) were in the same range.

The aerobic bacterial count of the samples purchased from Rimi market were higher than those of the control. Red Chili (*Jan yaji*) had the highest aerobic mesophilic count. This may be due to the fact that garlic and locust beans present in the other types of *yaji* are thought to have some antimicrobial effect (Carlson and Ensley, 2003). The fungal counts of the purchased yaji were also higher than those of the control. Out of the purchased samples, garlic-based *yaji* had the highest fungal count.

The high frequency of *Staphylococcus aureus* is of concern, since it has been implicated in foodborne illnesses like diarrhea and food poisoning due to their ability to produce enterotoxin.

The high coliform count and presence of *E.coli* and *Salmonella* are indicators of poor hygienic practices during preparation and handling. Some strains of *E.coli* and *Salmonella* are also capable ofcausing diarrhea, gastrointestinal illness and neurologic infections (Ugbogu *et al*., 2018).

The presence of fungal species in *yaji*samples were also reported by Ugbodu *et* al., (2018). The fungal species isolated from some of the samples includes *Fusarium* specie, *Rhizopus* specie and *Aspergillus* specie. The presence of these moulds in *yaji* samples may result in the production of toxic substances such as aflatoxinswhich could lead to serious health hazards of the consumers (Adebesin *et al.,* 2001).

CONCLUSION AND RECOMMENDATION

The study provides information on the proximate contents of *yaji* (spiced pepper mixture) and indicates that the different types of *yaji* sold in the study area have some nutritional benefits as they were found to be rich in carbohydrate, proteins and lipid which are essential to body mass development in humans. The study shows that the different combinations of *yaji* samples are contaminated with bacteria and fungi, exhibiting high microbial counts thereby posing public health threat. The study identifies the need for improvement in the preparation of *yaji* samples sold within Kano metropolis, such that they will become microbiologically safe for human consumption. Small and large scale producers of *yaji* should be encouraged to adopt the use of labels that indicates vital information such as nutritional content, recommendation for storage and expiry date as this can help in understanding the worth and benefits of *yaji.*

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