MICROBIOLOGICAL AND NUTRITIONAL ASSESSMENT OF 'NCHA IWU' (EDIBLE EMULSION) VENDED IN SELECTED MARKETS IN UMUNNEOCHI LGA, ABIA STATE, NIGERIA

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ABSTRACT

'Ncha iwu' is commonly used as a sauce in the preparation of ready-to-eat African salad, a wellknown delicacy among the Igbo tribe in Nigeria. A total number of fifteen (15) samples of 'ncha iwu' were randomly purchased from three (3) vendors in Orie Ngodo and Eke Eziama market in Umunneochi Local Government Area of Abia state. The microbiological quality, proximate composition, and mineral content of the samples were determined using Standard Methods. 'Ncha iwu' prepared in the laboratory served as the control. The results showed that total bacterial, Staphylococcal, Coliform and fungal counts of the samples ranged from 3.81-4.37, 2.0-3.58, 3.0-3.8, and 2.0-3.0 log₁₀ CFU/g, respectively. The bacterial species isolated and frequency of occurrence were Staphylococcus aureus (28 %), Klebsiella spp. (18 %), Pseudomonas spp. (18 %), Escherichia coli (18 %) while the fungal species were Penicillium spp. (50 %), Aspergillus spp. (40 %), and Candida spp. (10%). Proximate analysis indicated that 'ncha iwu' contains ash (63.93%), moisture (15.88 %), fibre (8.99 %), lipid (7.70 %), protein (3.50 %) and carbohydrate (0.0 %) while minerals content include Fe (3.679 mg/g), Mg (1.055 mg/g), K (1.054 mg/g), Ca (1.030 mg/g), Mn (0.469 mg/g) and Na (0.222 mg/g). Although 'ncha iwu' contains beneficial nutrients, majority of the commercial samples were contaminated with potential pathogenic microorganisms which is a public health concern unlike the control. This is as a result of poor handling by the producers and vendors. Good hygienic practices by the handlers, proper packaging and storage of 'ncha iwu' will reduce the microbial contamination of the product. Hence, adequate public health awareness campaign, training on food safety, sanitation and hygenic practices should be provided to the local processors and vendors of 'ncha iwu'.

Keywords: Palm bunch ash, Ncha iwu, production, microbiological quality, proximate analysis

INTRODUCTION

There are many local dishes consumed by millions of Nigerians depending on their culture, tribe, and geographical location irrespective of social status. One of the symbols of heritage of the people is traditional foods (Akinola *et al.*, 2020). Majority of the indigenous foods are fermented, often without chemical additives are delicious, nutritious, and beneficial to human health. The ingredients required to prepare traditional foods are sourced from affordable and abundant local materials (Uzogara *et al.*, 1990; Okeke *et al.*, 2008, 2009; Adesulu and Awojobi, 2014).

Palm bunch ash (PBA) obtained after burning empty palm fruit bunch is used in preparing 'ncha iwu' or 'ngu' as also called in some places. It is an edible sauce popular among the Igbo tribe that dominate the eastern part of Nigeria. Due to alkalinity of palm bunch, it is usually used to prepare local dishes (Ikezu et al., 2020). 'Ngu' also referred as African salad sauce is supposedly a non-purgative substance used in preparing palm oil and African salad. This exotic delicacy is a rich source of protein, carbohydrate, vitamins and minerals (Emelike and Akusu, 2018). Affordability, availability and preference based on taste influences the choice of ingredients used in preparing African salad. It is eaten alone or in combination with other snacks such as palm kernel, coconut and groundnut. African salad is served as a special cuisine during traditional festival (Emelike and Akusu, 2018). Sometimes, 'ngu' is used to substitute trona popularly known as 'kaun' that functions as a food additive and tenderizer in the preparation of African salad (Oranusi et al., 2013; Okoye et al., 2016).

Residents of some local communities in the Eastern part of Nigeria make use of palm bunch generally regarded as a waste after palm fruits have been removed to produce 'ncha'. This product is an edible emulsion base (a mild soap). In addition to being one of the ingredients for making African salad, the water and oil emulsion known as 'ncha' is also used in the preparation and consumption of bitter yam and oil bean that have been processed. Emulsions of edible fats and oils such as whipped dessert to pings, peanut butter and ice creams are sauces just like 'ncha'. Addition of the sauce to food products change its physical form. The taste of the food product will also change because it coats the tongue with emulsified oil and creates a mouth feel (Uzodinma et al., 2014).

Traditionally, the preparation of '*ngu*' involves the use of leaves namely *Kola ntida*,

Sida acuta ('iheaha'), Jatropha curcas ('kpokoko'), and Corchorus olitorius ('udeala ekpo') in addition to empty fruit bunch and palm oil sludge (Nasrin *et al.*, 2008). An emulsion of water and oil known as '*ngu*' is prepared using the filtrate obtained from plant ash mixed with water. The mixture is brownish. A slippery feeling noticed with the fingers suggests that the mixture is alkaline and product of saponification reaction (Ntukidem *et al.*, 2020).

Burning of empty fruit bunch which result in bunch (PBA) palm ash constitute approximately 6.5 % of the bunch by weight. About 30-40 % K₂O is found in PBA. Therefore, it can be used as a potassium fertilizer. Also contained in PBA is varying phosphorus. amount of calcium. and magnesium. In agriculture, PBA can be used as a liming material which increases soil fertility and pH. It also helps crops such as cassava and maize to absorb nutrients (Okoye et al., 2016). Traditionally, PBA is used to prepare edible delicacies such as 'otong' popular among the people of Annang, Akwa Ibom State. Other traditional foods prepared using plant ash are 'ighu', 'ugba', 'isiewu and nkwobi' (Udoetok, 2012; Ntukidem et al., 2020).

Several studies have been carried out on the materials used in preparing 'ngu' which include Corchorus olitorius, among others. Conchorus olitorius is known as jute mallow and 'ewedu' in English and Yoruba language, respectively. This leafy vegetable is common among the Yoruba tribe. In southeastern part of Nigeria, it is called 'ahihara' (Olusanya et al., 2018; Orieke et al., 2019). The leaves are a rich source of minerals, vitamins B1, B2, C and E, β -carotene and other carotenes. It also contains fatty acids, dietary fibre and protein (Adediran et al., 2015; Ali et al., 2019). Ethnomedical practice make use of the leaves to treat malaria, enteritis, fever, gonorrhea, tumors, pectoral pains, ache and pain (Adedosu *et al.*, 2015). It possesses antioxidant, antifungal, anti-inflammatory and antibacterial properties. Making use of the

cold infusion of *C*, *olitorius* is believed to restore appetite and strength (Adediran *et al.*, 2015; Yagagsa *et al.*, 2016).

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The leaves from the plant *J. curcas* are another ingredient used in preparing '*ngu*'. It is a rich source of minerals (Primandari *et al.*, 2018). *Jatropha curcas* is used in traditional medicine. The leaves help in the treatment of malaria, mouth infections, rheumatic, lymphocytic leukemia, guinea worm sores, muscular pains, diarrhea, dysentery and colic. A decoction of the leaves helps to fight cough. It also help to stimulate lactation (Prasad *et al.*, 2012; Patil *et al.*, 2013).

Preparation of 'ngu' also involves the use of C. nitida leaves. The health benefits, chemical composition and usefulness of C. nitida seeds have been researched extensively (Ojo et al., 2010; Amadi and Nwachukwu, 2020). However, the leaves of C. nitida appear not have attracted a lot of research interest comparable with the seeds. According to Kanoma et al. (2014), a tonic prepared using the leaves of *C. nitida* function as a therapy for cough, diarrhea, dysentery, chest complaints and vomiting. Studies have identified the presence of chemical constituents which include alkaloids. tannins, glycosides. flavonoids, saponins, steroids and reducing sugars in C. nitida leaves (Kanoma et al., 2014).

Sida acuta commonly known as wire weed is another leaf used in the preparation of 'ngu'. It is a rich source of protein, ash, vitamins, minerals, and energy (Shittu and Alagbe, 2020). C. acuta is used in folk medicine. The leaves possess antibacterial, antiprotozoal, antifungal, and antiprotozoal properties. It is used as antiulcer, demulcent and diuretic. Africans use the leaves as an abortifacient. Gingelly oil, also referred as sesame oil boiled with the leaves of *C. acuta* is applied on testicular swellings and elephantiasis as well as help quicken suppuration (Singh and Vishwavidyalaya, 2018).

Preparation of 'ngu' takes place in households often in an unhygienic environment. This practice exposes the product to microbial contamination which could pose public health risk. The fact that 'ngu' is not subjected to heating before it is added to ready-to-eat African salad further increases the risk of consumers to food borne diseases. In recent times, 'ngu' is considered to be healthier than potash well known as cooking potash (akanwu). For that reason, 'ngu' seems to be gaining more popularity as a good substitute to 'akanwu' in the preparation of African salad. However, Okoye et al. (2016) cautioned against adding either of the materials in large quantity in food because of its toxicity. That notwithstanding, very limited studies have been carried out on the quality of 'ngu' sold in local markets. Uzodinma et al. (2014) produced and evaluated the quality of 'ncha' also known as 'ngu' and compared the result with the control (commercial product). The study did not report the genus and species of microorganisms found in 'ngu' as well as assess the quality of the product sold in the market. Therefore, this study is aimed at determining the microbiological quality. proximate composition, and mineral content of 'ngu' sold in local markets.

MATERIALS AND METHODS

Study area

The study area is Umunneochi Local Government Area, Abia State, Nigeria. A Google map showing the location is presented as Fig. 1.



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Fig. 1: Google map of the study area (Umunneochi).

Sample collection

A total of fifteen (15) samples of already processed '*ngu*' also known as '*ncha iwu*' were randomly purchased from Orie Ngodo market, Isuochi and Eke Eziama market, Nneato, both in Umunneochi Local Government Area, Abia State, Nigeria using sterile plastic bottles with tight cork. The samples were transported immediately to the Microbiology Laboratory, University of Port Harcourt, for analysis.

Preparation of 'ncha iwu' from oil palm waste

Ncha iwu' or *'ngu'* is locally prepared by indigenes of Umunneochi. The flowchart for the preparation of *'ncha iwu'* in the laboratory is presented in Fig. 2. The product was

prepared by sun-drying spikelets from oil palm bunch stalk of oil palm (Elaeis guineensis) empty fruit bunch. Thereafter, the spikelets were burnt to ashes along with green C. nitida leaves C. acuta, Jatropha curcas, Corchorus olitorius and Okro leaves and allowed to cool. The ash was properly sieved to remove debris. The sieved ash was thoroughly mixed with fresh palm oil sludge. The mixture was manually molded into a circular shape and burnt together with oil palm bunch stalk to red hot and allowed to cool. This was to totally dehydrate every available moisture. Find attached, the pictorial representations (Plates 1a to 1h) of some of the raw materials used in the preparation of 'ncha iwu'. Thereafter, the hydrated 'ncha iwu' (Plate. 2) was used for further study.

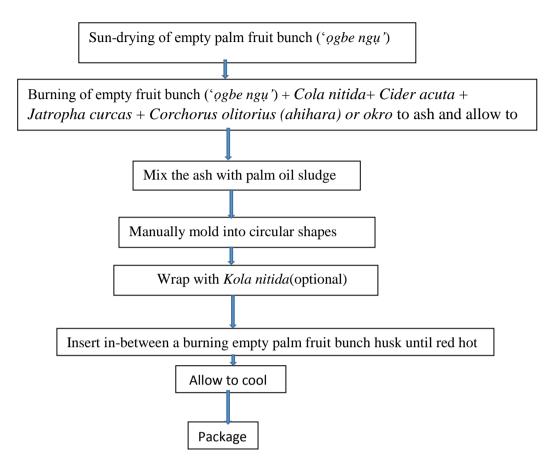


Fig. 2: Flow chart for the preparation of 'ncha iwu'.

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Plate 1a. Oil palm (*Elaeis guineensis*) empty fruit bunch (*Aghiriya akwu*)



Plate 1b. Oil palm bunch stalk(*Ogbe ngų*)



Plate 1c. Spikelets



Plate 1d.Jatropha curcas



Plate 1g. Cola nitida



Plate 1e. Cider acuta (ijikere)



Plate 1h. *Abelmoschus esculentus* (Okroplant)



Plate 1f. *Qgbara ndu na elu* or *udo ocha*



Plate. 2. Prepared '*ncha iwu*' ('*ngu*')

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Serial dilution of the samples

A homogenate was prepared using one gram (1 g) of '*ncha iwu*'. Aseptically, the homogenate was transferred into 9 ml of sterile normal saline and homogenized for 30 seconds. Six (6) test tubes containing 9 ml of sterile saline were used to carry out 10- fold serial dilution. One millilitre (1ml) of the stock solution was aseptically transferred into the subsequent test tube containing 9 ml of sterile normal saline using a sterile pipette. Using a sterile pipette for each transfer, the step was repeated until 10⁻⁶ dilution was reached.

Microbiological analysis

Isolation of bacteria from 'ncha iwu'

A sterile 1ml pipette was used to aseptically transfer 0.1 ml of the diluted sample from the dilution tubes labelled 10⁻⁴, 10⁻⁵ and 10⁻⁶ into sterile Petri dishes. A freshly prepared nutrient agar (NA) and de Man, Rogosa and Sharpe Agar (MRSA) was cooled to 47°C, swirled and then poured separately into the Petri dishes inoculated with the samples in duplicates. The inoculated Petri dishes were allowed to solidify. The MRSA plates were inverted and placed in an anaerobic jar, while the NA plates were incubated for 48 h at 37 °C. The colonies on each culture plate were counted with the aid of digital colony counter (Gallenkamp, England) and the number of colonies in the samples was calculated using the formula below. The result obtained is expressed as colony forming units per gram (CFU/g).

 $CFU/g = No. \text{ of colonies } x \frac{1}{\text{serial dilution}}$ $x \frac{1}{\text{dilution plated}}$

Purification of the bacterial isolates

Pure culture of the bacteria isolates was obtained by repeated subculturing on freshly prepared nutrient agar (NA). The isolates were preserved on slants kept inside a refrigerator maintained at 4°C until the isolates have been identified.

Identification of the bacterial isolates

Identification of the bacterial isolates was based on colonial morphology, microscopy and biochemical tests. The biochemical tests which include catalase, coagulase, oxidase, citrate, indole, urease, and triple sugar iron tests were performed on the bacterial isolates. Gram staining, spore formation, and sugar fermentation of the isolates were also carried out (Isu and Onyeagba, 2002; Shoaib et al., 2020).

Isolation of fungi from 'ncha iwu'

Aseptically, 0.1 ml of the diluted sample from the dilution tubes labelled 10^{-4} , 10^{-5} and 10^{-6} was poured inside the sterile Petri dishes in duplicates. Then, freshly prepared Sabouraud dextrose agar (SDA)added with ampicillin to inhibit the growth of bacteria which was allowed to cool to 47 °C was poured inside the Petri dishes. The inoculated plates were allowed to solidify and incubated for 7 days at room temperature $(28\pm2^{\circ}C)$. The colonies observed on the culture plates were counted with the aid of digital colony counter (Gallenkamp, England). Using the formula below, the number of colonies in the samples were calculated and the result obtained is expressed as colony forming units per gram (CFU/g).

 $CFU/g = No. \text{ of colonies } x \quad \frac{1}{\text{serial dilution}}$ $x \quad \frac{1}{\text{dilution plated}}$

Purification of the fungal isolates

Pure culture of fungal isolates was obtained after series of sub-culturing using freshly prepared Sabouraud dextrose agar (SDA).

Identification of the fungal isolates

Identification of the fungal isolates was carried out by adopting the needle mount method described by Ibrahim and Rahma (2009). The fungal spores were properly teased apart to ensure clear visibility. Staining of the fungal spores involved the use of cotton blue in lactophenol and examined microscopically using both the low and high power objective lens of the microscope. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys described by Cheesbrough (2004).

Proximate composition

The protein, moisture, total ash, crude lipid and fibre content of '*ncha iwu*' was determined using the method described by AOAC (2002). Carbohydrate content of the sample was determined by difference method.

Mineral content

The iron, manganese, calcium, potassium, sodium and magnesium content of '*ncha iwu*' was determined using the procedure described by Ire *et al.* (2020).

RESULTS

The total heterotrophic bacterial count (THBC) of '*ncha iwu*' sold in the markets and the controls showed that only 3 out of 16 samples of '*ncha iwu*' analyzed were contaminated with bacterial population with values between the range of 3.81-4.37 log₁₀CFU/g(Fig. 4). There was no THBC encountered in the control. An assessment of the Staphylococcal count of '*ncha iwu*' obtained from the markets and the control indicated that *Staphylococcus* spp. was present in 10 out of 16 samples of '*ncha iwu*' and the staphylococcal count was within the range of

2.0-3.58 \log_{10} CFU/g (Fig. 5). There was no staphylococcal count encountered in the control.

Total coliform count (TCC) of '*ncha iwu*' sold in the markets and the control is depicted in Fig.6. The result obtained showed that 4 out of 16 samples of the product were contaminated with coliforms. The TCC was within the range of 3.0-3.8 log₁₀CFU/g. Coliforms were not detected in the control. Figure 7 indicated the total fungal count (TFC) of '*ncha iwu*' sold in the markets and the control. The result obtained showed that fungi contaminated 10 out of 16 samples of '*ncha iwu*' and the values were within the range of 2.0-3.0 log₁₀CFU/g. The control had a TFC of 2.65 log₁₀CFU/g.

Proximate analysis showed that '*ncha iwu*' was rich in ash content (63.93 %), but carbohydrate was not detected (Table 1). Among the minerals reported in this study. (Table 2), sodium (0.222 mg/g) was the lowest in concentration whereas iron (3.679 mg/g) was the highest.

Following biochemical characterization, the bacterial isolates and frequency of occurrence were found to include *Staphylococcus aureus* (28 %), *Escherichia coli* (18 %), Lactic acid bacteria (18 %), *Klebsiella* spp. (18 %) and *Pseudomonas* spp. (18 %) (Fig. 8) while the fungal isolates were *Penicillium* spp. (50 %), *Aspergillus* spp. (40 %) and *Candida* spp. (10 %) (Fig. 9).

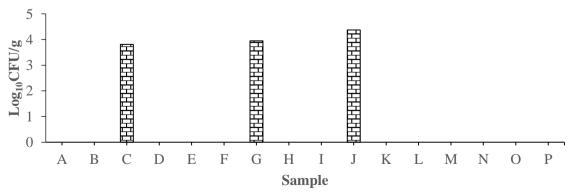
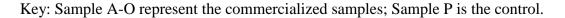


Fig. 4: Total heterotrophic bacterial count of 'ncha iwu'.



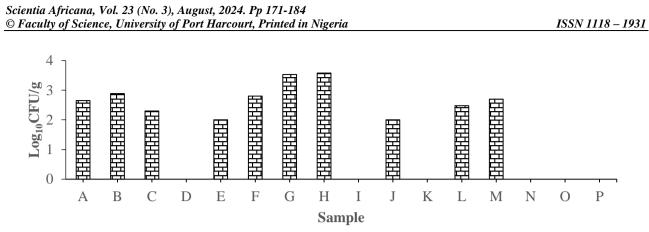
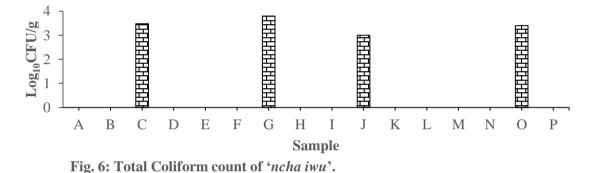


Fig. 5: Total Staphylococcal count of 'ncha iwu'

Key: Sample A-O represent the commercialized samples; Sample P is the control.



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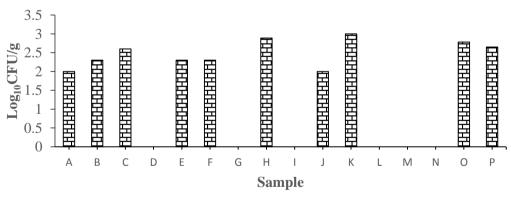
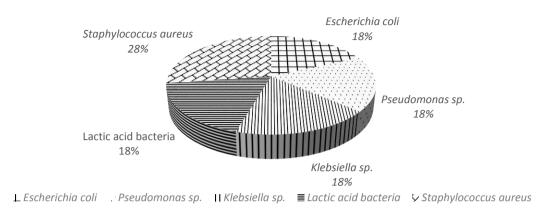


Fig. 7: Total fungal count of 'ncha iwu'.

Key: Sample A-O represent the commercialized samples; Sample P is the control.



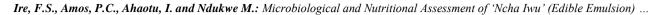


Fig. 8: Frequency of occurrence of bacterial isolates from 'ncha iwu'.

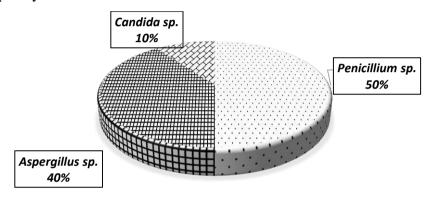


Fig. 9: Frequency of occurrence of fungal isolates from 'ncha iwu'.

Parameter	Quantity (%)
Lipid	7.70
Ash	63.93
Moisture	15.88
Carbohydrate	0.00
Protein	3.50
Fibre	8.99

Table 1:Proximate composition of 'ncha iwu'.

DISCUSSION

'Ncha iwu' obtained from the markets were contaminated with potential pathogenic microorganisms. Staphylococcus spp. and fungi contaminated most of the sampled products. Coliforms were found in fewer samples of 'ncha iwu' (4 out of 16 samples), but the population within the range of 3.0-3.8 log₁₀CFU/g was higher than that of Staphylococcus spp. and fungal species reported to be2.0-3.58 and 2.0 - 3.0

Table 2: Mineral c	content of '	ncha	iwu'	
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Minerals	Quantity (mg/g)
Iron	3.679
Manganese	0.469
Calcium	1.030
Potassium	1.054
Sodium	0.222
Magnesium	1.055

log₁₀CFU/g, respectively. This result raises a lot of concern on the possibility of feacal contamination of '*ncha iwu*' commonly produced in different households under unsanitary conditions especially in rural communities. The total heterotrophic bacterial count (THBC) of '*ncha iwu*' obtained from the market was within the range of 3.81-4.37 log₁₀CFU/g. The National Agency for Food and Drug Administration and Control (NAFDAC) specify that allowable limit of microbial load in food should not exceed 1 x 10^4 CFU (Edet *et al.*, 2020). Based on this specification, the THBC of all the samples of 'ncha iwu' obtained from the market including the control met the NAFDAC requirement with one exception. In a related study, Uzodinma et al. (2014) reported that total viable count and mold count of 'ncha iwu' also known as 'ngu' obtained from the market were 195 and 350 CFU/ml, respectively. Ntukidem et al. (2020) reported that microbial load of saponified dish well-known as 'otong' prepared using ash solution of oil palm bunches and unripe plantain peels as well as commercial alkaline solution (potash)that served as the control were within NAFDAC's acceptable limits.

Bacterial species identified from 'ncha iwu' were Staphylococcus aureus, Klebsiella spp., Pseudomonas spp. and Escherichia coli. Also identified from the product is a group of bacteria generally referred as lactic acid bacteria (LAB). The bacteria that had the highest percentage occurrence in the samples was Staphylococcus aureus (28%). This result could have serious health implications. Other bacterial species isolated from 'ncha iwu' obtained from the market had the same frequency of occurrence (18%). The presence of coliforms such as Escherichia coli in 'ncha iwu' is an indication of potential fecal contamination from humans and animals especially domestic pets. The source of Pseudomonas spp. in 'ncha iwu' sampled from the market could be from the soil and water. Contact of palm bunch ash used in preparing 'ncha iwu' with soil could have contaminated the product with *Pseudomonas* spp. There is possibility that lactic acid bacteria (LAB) isolated from 'ncha iwu' is from any of the leavesused in preparing the emulsion of edible fat 'ncha iwu'. Bamidele et al. (2011; 2016) isolated LAB from salad vegetables. Edet et al. (2020) identified four (4) bacterial species and ten (10) fungal species from saponified dishes prepared using ash solutions of oil palm bunches and unripe plantain peels known as 'otong'. The result is substantially in agreement with the findings from this study.

Fungi isolated from '*ncha iwu*' sold in the markets were *Penicillium* spp. (50 %), *Aspergillus* spp. (40 %) and *Candida* spp. (10 %). Notably, *Penicillium* spp. was the only fungal specie isolated from the control. These fungi species could be from dust, air, packaging material, environment and leaves used in the preparation of '*ncha iwu*'. Ihejirika *et al.* (2014) and Osawaru *et al.* (2013) isolated different fungi species from the leaves of *Jatropha curcas* and *Corchorus olitorius*, respectively.

'Ncha iwu' sampled from the markets had a high ash content (63.93%). Ash content of 'ncha iwu' could be largely attributed to palm bunch ash. According to Ikezu et al. (2020), the ash content of palm bunch is 2.91 %. Generally, the ash content of a food product is an indication of the amount of minerals it contains. Uzodinma et al. (2014) reported that ash content of 'ncha' (commercial product) also known as 'ngu' is 23.90 %. The crude protein content (3.50 %) of 'ncha iwu' is attributed to plant protein. Low lipid content (7.7%) of 'ncha iwu' could be attributed to the palm sludge used in preparing 'ncha iwu'. Carbohydrate was not detected in 'ncha iwu'. This result implies that '*ncha iwu*' is not a rich source of energy for the human body. However, incorporating 'ncha iwu' into African salad rich in carbohydrate (22.55 %) is most likely to counter the effect of low carbohydrate in 'ncha iwu' (Emelike and Akusu, 2018). It could be that fibrous nature of empty fruit bunch used in preparing 'ncha iwu' contributed significantly towards the crude fibre content (8.99%) of the product (Razali et al., 2012). Moisture content (15.88 %) of 'ncha iwu' reported in this study is marginally high. According to Ikezu et al. (2020), the moisture content of palm bunch is 53.77 %. It is most likely that igniting the palm bunch which resulted in palm bunch ash drastically reduced its moisture content.

This study has shown that '*ncha iwu*' is a rich source of minerals. The use of '*ncha iwu*' as African salad dressing could provide minerals beneficial to the human body. The result obtained from this study shows that iron, magnesium, potassium, calcium, manganese and sodium content of 'ngu' was 3.679, 1.055, 1.054, 1.030, 0.469 and 0.222 mg/g, respectively. It is most likely that minerals in 'ncha iwu' is largely from the palm bunch ash used in preparing the product (Eremrena and Mensah, 2017). The iron content in 'ncha iwu' was higher than other minerals analyzed in the product, whereas sodium was the least. Thus, 'ncha iwu' is regarded as a healthy substitute to potash as edible emulsifier for the preparation of African salad ('Abacha' or 'Iwu') and other local foods.

CONCLUSION

Most of the samples of 'ncha iwu' obtained from the markets were contaminated with pathogenic microorganisms potential compared to the laboratory prepared product. Among the bacterial and fungal isolates encountered in 'ncha iwu', Staphylococcus aureus and Penicillium spp. had the highest percentage occurrence, respectively. Ash content of 'ncha iwu' was quite high indicating high level of minerals whereas carbohydrate was not detected. Microbiological quality of 'ncha iwu' produced in the laboratory is better than the commercialized samples. Therefore, 'ncha iwu' should be hygienically prepared, packaged and stored to avoid exposing the edible emulsion to microbial contamination.

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