Acta Scientific Pharmacology

Volume 3 Issue 7 July 2022

Research Article

Antimicrobial Potential of Uda (*Xylopia aethiopica*) and Uziza (*Piper guineenses*) on Akamu Paste Prepared from White and Yellow Maize for Postpartum Mothers and Sensorial Properties of their Gruel

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Received: May 13, 2022

Published: June 20, 2022

Abstract

In some localities in Nigeria, even till today, antibiotics are not used after delivery due to their common traditional believes, instead hot pepper soups prepared from uda and uziza. Acceptability becomes a problem to some mothers due to arbitrary concentrations being prepared, hence their inclusion in akamu paste. Spiced akamu paste samples were prepared in two batched from white and yellow maize. One batch was by fermenting separately white and yellow maize with both spices and the other by adding them at the point of milling the steeped maize. Pastes from fermented white and yellow maize without spices served as controls. Microbial load of akamu paste samples from both white and yellow maize and the acceptability characteristics of their gruel were determined with standard analytical methods. The results showed that the bacterial load count of spiced akamu paste samples ranged from 5-10x10^7 with control 50-60x10^7, 3-7x10^7 with control 44-48x10^7, and 2-4x10^7 with control 32-35 respectively for dilutions for 10^{-1}, 10^{-2} and 10^{-3}. The fungal load count of the entire spiced akamu paste samples were too few to count while their controls ranged respectively 5.0-6.0x10^7, 4.4-4.8x10^7 and 3.5-3.8x10^7 for dilutions 10^{-1}, 10^{-2} and 10^{-3}. The paste from white maize fermented with uziza was the best preferred (6.20) which is liked slightly while that from yellow maize fermented with uziza was the least preferred (2.80) which falls between the range of dislike very much and like moderately in the 9-point Hedonic scale. Acceptability could be improved by reducing spice concentration used.

Keywords: Antimicrobial Potentials; Uda; Uziza; White Maize; Yellow Maize

Introduction

Pap (akamu or ogi) is a local generic name for semisolid food made from cereals (commonly sorghum, maize and millet). Pap is a staple food in most parts of African countries with different names and preparation methods depending on the locality [1]. It is called akamu by the Igbos and ogi by Yorubas The ones made specifically from sorghum are referred to as ogi-baba. The traditional preparation of akamu involves soaking of dried white or yellow maize in water for 24-72 h followed by wet milling and sieving to remove bran, hulls and germs which are discarded as animal feed [2]. During long steeping period, fermentation takes place to reduce the pH and modify the maize starch that makes the akamu what it is. The common fermenting bacteria include the species of *Leuconostoc, Lactobacillus, streptococcus, Pediococcus, micrococcus* and *Bacillus.* The fungal genera are mainly representatives of *Apergillus, Paecilomyceium, Penicillium, Cladosporium, Fusarium* and *Trichothecium* whereas the most common fermenting yeast species is Saccharomyces, which contributes to alcoholic fermentation [2,3].

The colour of the akamu paste depends on the cereal used in the preparation. Where yellow maize is used, it has a light yellow appearance as against creamy or white appearance when white maize is used [4]. When the paste is dissolved with cool water to form light consistency and gelatinized with hot water (100°C), it turns into a semi-solid gruel (akamu) which can be taken alone or enriched with ingredients like beverages, milk, and soymilk among others according to choice. Enriched gruel is usually served as weaning food for infants, as breakfast for children and convenient meal for its convalescence [4]. The gruel is served with protein rich foods such as beans [5], bread, akara or fried plantain. It is often taken by nursing mothers as it encourages breast milk supply, easily digestible and a good vehicle for food nutrients for infants, elderly and sick people [6].

Akamu paste is deficient in nutrient due to inevitable processing losses, hence the enrichment of the gruel before consumption. Various studies have been carried out to improve the nutritional value by fortification [7] and formulation with nutrient dense legumes like soybean [8]. Akamu paste is fairly acidic (pH 4.8), which tends to inhibit the growth of some bacteria but liable to spoil due to high moisture content [9]. Its spoilage however, is enhanced by some extrinsic factors like storage temperature among others. Extension of its shelf life can be achieved by refrigeration, freezing and drying (dehydration) to reduce the availability of moisture and microbial load. It can also be preserved by plant materials such as *Xylopia aethiopica* (*uda*) and *Piper guineenses* (*uziza*) because they possess active ingredients which are either microbistatic or microbicidal [10]. These plant materials are mainly used as spices, stimulants and flavouring agents on some foods in South Eastern states in Nigeria depending on their natural medicinal values.

Xylopia aethiopica which is called uda by the Igbos belongs to the family Annonaceae and is used mainly as spice and in traditional medicine [11]. Its seeds extracts offer a therapeutic role in disease prevention due to antioxidant properties of their parts which contain both synthetic and natural compounds that scavenge free radicals and inhibit their oxidation processes. Antioxidant properties of therapeutic plants are preferred to synthetic compounds which are associated with side effects and toxic properties that weaken health [12]. The role of the antioxidants is to neutralize the excess free radicals, to protect the cells against their toxic effects and contribute to disease prevention by donating electron to the free radical to become stable. The seed extract assist in the contraction of the uterus in post-patum women [13]. Uda contains cardiac glycoside, flavonoids, phenol, phlobotannins, anthroquinones, tannins and steroids [14]. The fruits can also be crushed and mixed with Shea butter and used as body creams, cosmetic products. The green mature fruit which becomes brown-black in colour after drying is commonly used as spices [15].

Piper guineense belongs to the family Piperaceae. It is a West African spice plant commonly called "Ashanti pepper", uziza in Igbo and iyere in Yoruba. Other common names are black pepper, Benin pepper, Guinea pepper and false cubeb [16]. It has high commercial, economical, and medicinal values. Extracts from its leaves, roots and seeds are used for the treatment of bronchitis, gastrointestinal disorders and rheumatism [17]. Uziza has high mineral content such as calcium, zinc, magnesium, copper and potassium in the vegetable [18] with appreciable protein and carbohydrate contents. More so, uziza contains vitamin C, vitamin A and traces of vitamin B1 and B2, vitamin E [19]. This study explored the effects of spicing akamu paste with uda and uziza on their microbial loads and the acceptability levels of the gruel prepared from them.

Materials and Methods

Source of raw materials

Uda (Figure 1), uziza (Figure 2), white (Figure 3) and yellow maize (Figure 4) were purchased from Ubani main market in Umuahia North Local Government of Abia State, Nigeria

Figure 1: Uda Seeds.

Figure 2: Uziza seeds.

Figure 3: White maize.

Figure 4: Yellow maize.

Sample preparations

Preparation of akamu paste fermenting maize with the spices

The grains were sorted to remove immature ones, stones and other unwanted materials. Same quantity of white and yellow maize was separately steeped (100g maize/5 g of each spice) in a stainless basin with clean tap water for 72h without changing the water. Thereafter, each batch was washed, wet milled with a locally fabricated attrition mill and their slurries sieved using a sterile muslin cloth. Their filtrates were allowed to stand for 24h to sediment so as to achieve sufficient draining of the water. Their sediments were separately packed into double layer muslin cloth and dewatered manually by squeezing or placing a heavy weight on it to filter of some water through the muslin cloth's eyelets until water ceases to come out. Semi solid paste obtained is called akamu paste which were packed in odour-free airtight containers and stored at refrigeration temperature until needed.

Preparation of akamu paste from fermented maize with ground spices added at the point of milling

Same process as above was carried out here except that the spices were not fermented with the maize, rather were added ground at the point of milling the maize. The pastes obtained were also store at refrigeration temperature till needed.

Fermented corn only

The same process was repeated here but without the spices which served as controls. Both pastes from white and yellow maize obtained were also stored at refrigeration temperature.

Preparation of gruel for sensory analysis

Each akamu paste sample was dissolved (10g paste: 10 ml water) in cool water to obtain a moderate light consistency which was gelatinized by pouring boiled (100^{o}C) water (50 ml) to obtain the gruel.

Microbiological Analysis of the Paste Samples

Total bacterial and fungal counts were enumerated [20]. Tenfold serial dilution was made. One gram of each spiced *akamu* paste sample was aseptically dispensed into three test tubes containing normal saline. Thereafter, each tube was mixed thoroughly by shaking and 1 ml of each of the mixture was aseptically pipetted into another set of three test tubes containing each 9 ml normal saline to give 10^{-1} dilutions. The same process was repeated two more times to obtain 10^{-2} and 10^{-3} dilutions respectively. The same procedure was repeated for the controls. The test tubes containing the dilutions were each stuffed with cotton wool and covered with foil. Exactly 0.1 ml aliquot of each dilution was inoculated into each petri-dish containing nutrient agar and potato Dextrose agar for the determination of total cultivable bacteria and fungi respectively [21]. The plates were incubated at 30°C for 34h thereafter both discrete colonies of bacteria and fungi of each plate yield were enumerated and expressed in colony units.

Sensory scores

The acceptability levels of the gruels were evaluated subjectively with 30 untrained panellists drawn from the students in the Food Science and Technology Department aged between 18-22 y that are used to drinking akamu gruel. They were presented with the samples warm and coded in similar plates in a well-lighted room along with a bottle of water each to rinse their mouth before and after each tasting. They were ask to taste each sample and score them according to 9-point Hedonic scale where 1 is like extremely, 9 dislike extremely and 5 neither like nor dislike. Attributes evaluated were colour, taste, consistency and general acceptability.

Data analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) while the Duncan Multiple Range Test (DMRT) was used to compare means at 95% confidence level ($p < 0.05$). All statistical analysis was done using the SPSS version 21.0 software.

Results and Discussions

Total bacterial counts

Total bacterial counts of the akamu paste samples as presented in table 1 revealed that the colony counts of the entire un-spiced akamu paste were higher than the spiced regardless of spice and maize types, spicing technique and serial dilution. This could be attributed to the antimicrobial properties of both spices [10] due to their phenolics content [22]. This notwithstanding, the bacteria counts of both spiced and un-spiced paste decreased with increase in dilution factors. This could be due to the dilution that also diluted the bacteria load present in the pastes. Un-spiced fermented yellow maize (YM) paste had higher bacterial load (35.0-60.0 x 10^7) than 32.0-50 x 10^7 from white maize (WM) in all the dilutions. This could be because of maize variety depending on the component. This result is at variant with the report on ogi paste from three varieties of maize [2], may be due to maize variety, soil chemistry of the planting soil, level of contamination and the preparation technique.

With WM, spicing at the point of milling with uda had the highest bacterial count compared to the rest, followed by fermenting uda in all the dilution levels. There were no difference in bacterial load of all uziza spiced WM in all the dilutions regardless of spicing techniques. Higher count implied that during milling, there was not enough resident time for the uda to affect its antiseptic property on the bacteria unlike when fermented with it. Conversely, lower and no bacterial count variation in all uziza spiced pastes could be that its antiseptic activity is higher than that of uda.

Colony counts in each dilution			
Sam[less	10^{-1}	10^{-2}	10^{-3}
F(WM+Uda)	9.0	4.0	2.0
F(WM)+G(Uda)	10.0	4.0	4.0
F(WM+Uziza)	5.0	3.0	2.0
F(WM)+G(Uziza)	5.0	3.0	2.0
F(YM+Uda)	7.0	3.0	2.0
F(YM)+G(Uda)	6.0	4.0	3.0
F(YM+Uziza)	8.0	7.0	4.0
F(YM)+G(Uziza)	10.0	6.0	4.0
FWM	50.0	44.0	32.0
FYM	60.0	48.0	35.0

Table 1: Total bacteria count of spiced and un-spiced akamu paste samples (x 107 Cfu/ml).

Values are means triplicate determinations ± standard deviation. Values in the same column with different superscript are significantly different ($p < 0.05$) from each other. F(WM+Uda) = Fermented white corn and uda. F(WM)+G(Uda) = Fermented white maize and ground uda F(WM+Uziza) = Fermented white corn and uziza. F(WM)+G(Uziza) = Fermented white corn and ground uziza. F(YM+Uda) = Fermented yellow corn and uda. F(YM)+G(Uda) = Fermented yellow corn and ground Uda. F(YM+Uziza) = Fermented yellow corn and uziza. F(YM)+G(Uziza) = Fermented yellow corn and ground uziza. FWM = Fermented white corn only. FYM = Fermented yellow corn only.

With YM, spicing with uziza at the point of milling is higher only at 10^{-1} dilution which also implied that fermenting with uziza like with uda in WM is more effective in reducing the bacterial count than milling with it. Fermenting YM with uda is more effective in reducing bacterial count than with WM which point to as a better antibacterial effect than uziza in reducing the bacterial count. Similarly, milling YM with uda decreased the bacterial load more than with WM thereby projecting milling with uda as more effective in reducing the higher microbial count of YM. Uziza is more effective in reducing microbial count of WM when fermented than with YM while grinding WM with uziza is more effective than with YM. Therefore, spice and maize types as well as spicing techniques decide the bacterial count of the spiced akamu pastes.

Total fungal count

The total fungal count of the akamu paste samples as resented in table showed that the counts of the entire spiced samples for both yellow and white maize in all the dilutions were too few to count (TFTC) unlike the un-spiced. This could be due to fairly acidic content of the paste (4.8) as higher pH (above 4.5) favours bacteria growth [23] unlike fungi that are favoured by higher pH [24]. However, there was no notice of whitish deposits due to fungi growth as fungi are surface growing organisms. Besides, the spices may have had more effects on the fungi than on the bacteria. Similar decreasing fungi growth by acidic medium had been reported [24] on tomatoes. Aside from this, un-spiced FYM paste had higher fungi count than FWM paste counterpart in all dilutions except at 10^{-3} level. Both pastes from FWM and FYM fungi counts also decreased as the serial dilution decreased probably due to decrease in the fungi load. This is an indication that FYM had more fungi load just like in bacteria which also validated the maize type as a function of microbial load.

Sensory properties

The results are presented in table 2.

Colony counts in each dilution			
Samples	**10^{-1}**	**10^{-2}**	**10^{-3}**
F(WM+Uda)	TFTC	TFTC	TFTC
F(WM)+G(Uda)	TFTC	TFTC	TFTC
F(WM+Uziza)	TFTC	TFTC	TFTC
F(WM)+G(Uziza)	TFTC	TFTC	TFTC
F(YM+Uda)	TFTC	TFTC	TFTC
F(YM)+G(Uda)	TFTC	TFTC	TFTC
F(YM+Uziza)	TFTC	TFTC	TFTC
F(YM)+G(Uziza)	TFTC	TFTC	TFTC
FWM	$5.0x10^7$	$4.4x10^7$	$3.8x10^7$
FYM	$6.0x10^7$	$4.8x10^7$	$3.5x10^7$

Table 2: Total fungal count of spiced and un-spiced akamu paste samples (Cfu/ml).

Values are means of triplicate determinations ± standard deviation. Values in the same column with different superscript are significantly different ($p < 0.05$) from each other. F(WM+Uda = Fermented white maize and uda. F(WM)+G(Uda) = Fermented white maize and ground uda. F(WM+Uziza) = Fermented white maize and uziza. F(WM)+G(Uziza) = Fermented white maize and ground uziza. F(YM+Uda) = Fermented yellow maize and uda. F(YM)+G(Uda) = Fermented yellow maize and ground uda F(YM+Uziza) = Fermented yellow maize and uziza. F(YM)+G(Uziza) = Fermented yellow maize and ground uziza. FWM = Fermented white maize only. FYM = Fermented yell ow naize only. TFTC = Too few to count.

Appearance

Appearance enhances consumer's attraction as they use what they see to accept or reject food [25]. This is an aspect of sensory evaluation that is a function of desirability. Gruels prepared with YM had significant ($p < 0.05$) better appearance (6.00) than 5.00 from white maize. This may be the major reason why the appearance of the gruels prepared from spiced YM were scored significantly ($p < 0.05$) higher (3.30-5.40 when spiced with uda, and 1.30-2.00 when fermented with uziza than the gruel from the white maize counterparts except when added during milling (3.80). Higher appearance score of uda spiced YM gruel could be attributed to the carotene content which gave them an appealing light yellow colour more than the white maize. This is evident in the significant ($p < 0.05$) higher appearance of the un-spiced YM gruel (6.00) than the WM (5.00). Besides, uziza contained more tannin than uda which may have caused some levels of undesirable browning and pigmentation during milling with ground uziza [26]. Despite these, spice type, irrespective of the spicing technique never improved gruel appearance as all their appearance scores were lower than their respective controls. But addition of ground spices at the point of milling had higher ratings than fermenting with them which could be due to uniform mixing resulting from higher surface area of the ground spices and lack of component complex interactions that may take place during fermentation. Therefore, improvement in gruel appearance depends on maize and spice types as well as spicing technique employed.

Taste

There was no significant ($p < 0.05$) taste variation between spiced and un-spiced gruels from both maize varieties, but the scores of the un-spiced were significantly ($p < 0.05$) higher than the entire spiced gruels. The taste scores of gruel samples from WM spiced with uziza irrespective of maize types and spicing techniques (5.60-5.70) and 4.00-5.50 from YM were scored higher than those from uda spiced YM. Uziza may have improved gruel taste more than uda unlike in appearance. Spice and spicing techniques on WM never had any significant ($p < 0.05$) score variations which suggested that both spices may have imparted a similar taste to their gruel samples.

Consistency

There was no significant ($p < 005$) consistency variation between both un-spiced gruels, but that from YM (4.10) is slightly higher than 3.70 from WM, may be because of variety and variations in their amylose and amylopectin ratios [27]. The consistency score (5.90) of gruel from WM spiced with ground uziza at the point of milling is significantly ($p < 0.05$) higher than all spiced and un-spiced gruel samples. All the consistencies of YM spiced gruels with uda and uziza were significantly ($p < 0.05$) higher than that of their WM counterparts except when ground uziza was added to

YM at the point of milling. This may suggest that gruel consistency is a function of maize variety. Carotene and other components of YM may be the primary cause of variation. Gruel samples from all the spiced YM had no significant ($p < 0.05$) consistency variation compared with the controls, but their spiced WM counterparts were significantly ($p < 0.05$) lower except when uziza was added to WM at the point of milling (5.90). It is pertinent to note that despite higher consistency obtained with YM, WM spiced with uziza had the best consistency. Therefore, maize and spice types as well as spicing technique influence the gruel consistency.

General acceptability

There were no significant ($p < 0.05$) acceptability variations between the gruels from un-spiced akamu pastes from WM and YM maize despite the rating variations in appearance, taste and consistency. Also, there was no significant ($p < 0.05$) acceptability variations between all the spiced gruel from WM, the controls and that from when uda was fermented with YM. This notwithstanding, paste from WM spiced with grounded uziza at the point of milling was the best desired (6.20) which is ranked liked slightly in the 9-point Hedonic scale. This could be explained by higher ratings of two (taste and consistency) out of three rated attributes.

Samples	Appearance	Taste	Consistency	General Acceptability
FWC	$5.00^{bc} \pm 0.13$	$6.50^{a} \pm 0.32$	$3.70^{b} \pm 0.04$	$4.60^{b} \pm 0.12$
FYC	$6.00^{a} \pm 0.11$	$6.60^{a} \pm 0.12$	$4.10^{b} \pm 0.04$	$4.60^{b} \pm 0.06$
F(WC+Uda)	$1.10^{f} \pm 0.01$	$4.20^{bc} \pm 0.03$	$3.90^{b} \pm 0.03$	$4.30^{b} \pm 0.05$
F(WC)+G(Uda)	$4.60^{c} \pm 0.12$	$4.20^{bc} \pm 0.04$	$1.70^{c} \pm 0.04$	$4.60^{b} \pm 0.10$
F(WC+Uziza)	$1.00^{f} \pm 0.11$	$5.60^{ab} \pm 0.02$	$1.70^{c} \pm 0.05$	$4.60^{b} \pm 0.11$
F(WC)+G(Uziza)	$3.80^{d} \pm 0.08$	$5.70^{ab} \pm 0.09$	$5.90^{a} \pm 0.08$	$6.20^{a} \pm 0.13$
F(YC+Uda)	$3.30^{d} \pm 0.04$	$4.30^{bc} \pm 0.07$	$3.40^{b} \pm 0.11$	$4.30^{b} \pm 0.12$
F(YC)+G(Uda)	$5.40^{ab} \pm 0.03$	$4.40^{bc} \pm 0.05$	$4.00^{b} \pm 0.02$	$3.60^{bc} \pm 0.11$
F(YC+Uziza)	$1.30^{ef} \pm 0.09$	$4.00^{c} \pm 0.11$	$3.10^{b} \pm 0.11$	$2.80^{c} \pm 0.09$
F(YC)+G(Uziza)	$2.00^{e} \pm 0.11$	$5.50^{abc} \pm 0.13$	$3.10^{b} \pm 0.09$	$4.10^{bc} \pm 0.06$

Table 3: Sensory characteristics of spiced and un-spiced gruels**.**

Values are means of triplicate determinations ± standard deviation. Means in the same column with different superscript are significantly different at ($P < 0.05$). FWM-Fermented white maize only, FYM-Fermented yellow maize only, F(WM+Uda)-Fermented white maize and uda, F(WM)+G(Uda)-Fermented white maize and ground uda, F(WM+Uziza)-Fermented white maize and uziza, F(WM)+G(Uziza)-Fermented white maize and ground uziza, F(YM+Uda)-Fermented yellow maize and uda, F(YM)+G(Uda)-Fermented yellow maize and ground uda, F(YM+Uziza)-Fermented yellow maize and uziza and F(YM)+G(Uziza)-Fermented yellow maize and ground uziza.

Conclusion

Both uda (*Xylopia aethiopica*) and uziza (*Piper guineenses*) showed interesting antimicrobial properties which varied significantly with their concentrations. Significant count of bacteria per colony forming unit in the akamu paste depended more on the spice type and maize variety than on fermentation. From this research, spicing akamu paste with uda snd uziza for postpartum women is feasible when used appropriately. Akamu paste which is prone to microbial spoilage when stored at ambient condition may be spiced with these spices to extend their shelf life. Only gruel from uziza white maize at the point of milling was best preferred (6.20) than the rest which signified liked slightly, followed by those from white maize fermented with uziza and uda at the point of milling. All the spiced gruels from yellow maize were accepted lower than their while yellow maize fermented with uziza is the least (2.80).

Therefore, spicing white maize with uziza at the point of milling produced the most accepted gruel in this study which may be improved by reducing the spicing concentration used in this study. Spicing of akamu paste for post patunl women which is a function of spice and maize typed as well as spicing technique is a matter of choice.

Acknowledgement

The authors are grateful to the Department of Food Science and Technology for providing analytical space and reagents used.

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