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Effect of Ginger and Cloves Fortification on the Microbial, Proximate, Sensory and Bioactive Constituents of Pito

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ABSTRACT

Pito is a sorghum-based beverage widely consumed in Nigeria and some parts of Africa, but the quality of the beverage has not been well documented. This study therefore investigated the effect of fortification on the quality of fermented pito. Different samples of pito were prepared; 100% sorghum (control), 75% sorghum + 15% cloves + 10% ginger and 65% sorghum + 20% cloves + 15% ginger. The microbial, proximate, sensory and bioactive constituents of the pito samples were determined. Data was analyzed using ANOVA at $p < 0.05$. Highest bacterial, fungal and coliform counts were recorded in the100% sorghum pito. Among the isolates, *Saccharomyces cerevisiae* was the predominant with percentage occurrence ranging from 84.75 to 100% in 100% sorghum pito and 65% sorghum + 20% cloves +15% ginger fortified pito respectively. Highest fat, protein and fiber content was recorded in the pito produced with 75% sorghum + 15% cloves + 10% ginger. The tannin, alkaloids, flavonoids and phenol constituents of the 75% sorghum + 15% cloves + 10% ginger fortified pito are significantly higher ($p < 0.05$) than the rest of the pito samples. Moreover, the sensory scores with respect to colour, taste and overall acceptability of the 75% sorghum + 15% cloves + 10% ginger fortified pito was significantly higher ($p < 0.05$) than the rest of the pito samples. Fortifying sorghum with cloves and ginger at the right proportion during pito preparation will help in meeting the microbiological, nutritional, sensory quality and health needs of consumers.

Keywords: Beverages, Pito, *Saccharomyces cerevisiae*, Sorghum, *Syzygium*, *Zingiber officinale*

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INTRODUCTION

Sorghum beer (pito) is an alcoholic beverage usually produced from fermented sorghum. It is mainly produced in northern Ghana, parts of Nigeria, and other parts of West Africa. The beverage is produced on a small scale and household basis and can be served either warm or cold in calabash outside the producer's home, where benches are sometimes provided for the consumers (Zaukuu et al. 2016). Pito brewing is a vital source of income for low income earners in rural areas. In Nigeria, many women especially in rural areas are involved in small scale production of pito which has helped to alleviate poverty among the people. However, the production of pito for commercial purposes and its processing is highly prone to microbial contamination (Abimbola and Alagamba, 2020). Many microorganisms implicated in pito contamination may include bacteria such as *Escherichia coli*, *Salmonella* species, *Shigella* species, *Staphylococcus aureus* (Umaru et al. 2014) and fungi such as *Aspergillus flavus*, *Aspergillus niger,* and *Saccharomyces cerevisiae* (Clavijo et al. 2011).

According to Sefah-Dedeh et al. (1999), the production processes for pito involve malting, germination, drying, milling, malt extraction, boiling, cooling and fermentation. The final product (pito) is dark brown in colour, with a slight sweet-to-sour taste. Pito is usually consumed as a refreshing, nutritious beverage to quench hunger and taste. It is a good source of energy and contains significant levels of proteins (Ajiboye et al. 2014). The beverage also provides the consumers with active components of functional foods such as polyphenols, micronutrients and macronutrients that plays an important role in the prevention of diseases relating to metabolic imbalances including gastrointestinal disorders, inflammation, obesity, hypertension, type 2 diabetes as well as cancer (Pang et al. 2012). The presence of essential minerals such as calcium, magnesium and iron, which are responsible for body and tissue regulation have been reported in sorghum beer (Kolawole et al. 2018). Sorghum, the active ingredient in pito is a major source of antioxidants and phytochemical constituents, thus has an excellent health benefits (Dykes et al. 2009).

Food fortification is regarded as a very effective intervention process for the prevention of nutritionally deficient abnormalities (Bishai and Nalubola, 2002). Fortification of traditionally fermented food products was reported to increase the concentration and bioavailability of the nutritional content of the edible part of the plant food, especially cereals, to the levels that consistently exceed the inherent content (White and Broadly, 2005; Olayiwola et al. 2017). Ginger (*Zingiber officinale*) is a spice that was originally found in Asia, but has now been widely planted and distributed around the world. It is one of the most commonly consumed dietary condiments globally (Surh et al. 1999). The oily resin from the roots of ginger contains many bioactive compounds such as gingerol and zingerone, which are believed to be the primary ingredient responsible for its wide array of medicinal uses. Ginger is also high in antioxidants which helps to mop up free radicals, thereby preventing cell damage (Blomhoff, 2004). Cloves (*Syzygium aromaticum*, *Eugenia aromaticum* or *Eugenia caryophyllata*) has its origin from the Moluccas Islands and is actually known as Spice Island. The tree that creates the miracle of nature are commonly used in biryanis, pickles, salads, garam masala, and it is a common product found in the spice rack around the world (Milind and Deepa, 2011). Clove oil contain active constituents which possess antioxidant, antimicrobial, anti-diabetic, anti-inflammatory, anti-thrombotic, anesthetic, pain relieving and insect repellent properties. Eugenol is the major constituent responsible for the medicinal properties of the clove bud (Milind and Deepa, 2011).

The microbial, proximate and sensory quality of pito has been the focus of research in recent times (Abimbola and Alagamba, 2020). However, there is paucity of information regarding the effect of fortification on the microbial, proximate and sensory quality of the beverage. Moreover, the bioactive constituents of pito has not been well studied. This study therefore investigated the effect of ginger and cloves fortification on the microbial, proximate, sensory and bioactive constituents of sorghum beer, pito.

MATERIALS AND METHODS

Source of materials for pito production

Sorghum (red colour variety), ginger, cloves and a slimy material (Okra) were purchased from second market, Ifite Awka, Anambra State, Nigeria. They were placed in polyethene bags and transported to the microbiology laboratory of Nnamdi Azikiwe

University, Awka, for processing and preparation of pito.

Preparation of pito

The sorghum grains were cleaned by handpicking to remove stones and other debris. This was followed by soaking the grains in water for 48 hours, during which the steeping liquor was changed at 12 hours interval to prevent odour (Abimbola and Alagamba, 2020). The steeped grains are spread and allowed to germinate for 4 days in a basket lined with moistened banana leaves. The malted grains were sun dried and ground into powder using pestle and mortar. Dry ginger and cloves were also ground separately into powder using pestle and mortar. Different fortifications of ground sorghum with powdered ginger and cloves were prepared as follows: 500 g (100%) sorghum (unfortified) was set up as the control (sample A). Sample B contained 380 g (75%) sorghum) + 75 g (15%) clove + 50 g (10%) ginger. Sample C contained 325 g (65%) sorghum + 100 g (20%) clove + 75 g (15%) ginger. Each of the experimental set up were mixed thoroughly with 4 liters of water, followed by 1 liter of a slimy solution made out of sliced okra stem; which facilitated the sedimentation of the insoluble mash (Zaukuu et al. 2016). After sedimentation, the supernatant was decanted and the coarse particles was boiled for 30 minutes (with constant stirring) to gelatinize. After boiling, the supernatant was then mixed with the boiled coarse particles, allowed to cool and left to ferment for 24 hours. The mixture was then filtered using a fine mesh material to remove the coarse particles. The wort obtained was boiled for 30 minutes to concentrate and allowed to cool. A small quantity of the starter culture (sediment from previous brew) was added to the cooled concentrate and left to ferment for another 12 hours. The pito samples were stored in sterile bottles and analyzed within 24 to 48 hours of preparation.

Microbial analysis of the pito samples

The media used for microbial analysis include nutrient agar (NA) for total heterotrophic bacterial count and isolation, sabouraud dextrose agar (SDA) for total fungal count and isolation and eosin methylene blue (EMB) agar for total coliform count and isolation. The media were prepared according to the manufacturer's instruction. Stock solutions of the pito samples (A, B, C) were prepared by pipetting 10 ml each into 90 ml of sterile peptone water in a conical flask. A ten-fold serial dilution of the aliquot was carried out and 0.1 ml aliquot of the diluted samples was transferred in triplicate onto the surface of solidified NA, SDA and EMB agar plates. The plates were gently swirled and allowed to set for 10 minutes. The nutrient and EMB agar plates were incubated at 37^{o}C for 24 hours, while the SDA plates were incubated at 28^{o}C for 72 hours. At the end of the incubation period, colonies that developed were counted using digital colony counter (Gallenkamp England), and expressed as colony forming unit per milliliter (cfu/ml). Pure cultures of the isolates were obtained by sub culturing onto the respective agar plates and identified on the basis of colonial morphology, microscopic and biochemical characteristics (Cheesbrough, 2002; McLandsborough, 2004).

Proximate analysis of the pito samples

The proximate analysis of the pito samples was carried out at Docchy Analytical Laboratories and Environmental Services, Awka. The moisture content, total protein, ash, fat content and crude fiber of each pito sample was determined using the procedure outlined in the official methods of analysis of the Association of Analytical Chemists (AOAC, 2000). The total carbohydrate (%) was estimated by difference.

Sensory evaluation of the pito samples

The sensory evaluation of the pito samples for consumer acceptance and preference was carried our using a 9-point Hedonic scale; with sensory score 1 representing extremely dislike and 9 representing extremely like. A ten member panelists including students of the department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, were recruited and trained for the study. Sensory properties such as colour, flavour, aroma, taste and overall acceptability were evaluated and water was provided to the participants to rinse their mouth between evaluations.

Bioactive constituents of the pito samples

Percentage flavonoids was determined using the method of Boham and Kocipai (1974), while percentage phenols were estimated using the method of Harborn (1988). Percentage tannin was determined following the Follins-Dennis titration method as described by Pearson (1976). Percentage alkaloids were determined by the methods of AOAC (2000) and Maxwell et al. (1995).

Statistical analysis

Data were analyzed and presented as ± standard error of mean (SEM). The sensory scores as well as the proximate and bioactive constituents of the pito samples were subjected to one way analysis of variance (ANOVA), using SPSS software version 20. Means were separated using the least significant difference (LSD) and Duncan's multiple range test at $p < 0.05$.

RESULTS

Table 1 depicts the result of the microbial count of the pito samples within 24 to 48 hours of preparation. Highest bacterial and fungal counts of $7.3\pm0.17 \times 10^2$ cfu/ml and $2.9\pm0.10 \times 10^2$ cfu/ml was recorded in the pito samples produced with 100% sorghum (control), while the lowest bacterial and fungal count of $1.4\pm0.15 \times 10^2$ and $1.0\pm0.15 \times 10^2$ cfu/ml was recorded in the pito samples produced with 65% sorghum + 20% clove +15% ginger. However, minimal coliform count of $0.1\pm0.05 \times 10^2$ cfu/ml was recorded in the pito sample produced with 75% sorghum + 15% clove + 10% ginger, while zero coliform count was recorded in the pito sample produced with 65% sorghum + 20% cloves + 15% ginger. Moreover, highest coliform count of $0.5\pm0.11 \times 10^2$ cfu/ml was recorded in the pito sample produced with 100% sorghum.

Table 1 Total microbial count of the pito samples

Pito samples	NA ($\times 10^2$) cfu/ml	EMB agar ($\times 10^2$) cfu/ml	SDA ($\times 10^2$) cfu/ml
A	7.3±0.17	0,5±0.11	2.9±0.10
B	4.3±0.10	0.1±0.05	2.3±0.05
C	1.4±0.15	0.0±0.00	1.0±0.15

Values are mean ± SEM of three replicates. A = Unfortified pito (100% sorghum), B = 75% sorghum + 15% cloves + 10% ginger, C = 65% sorghum + 20% clove + 15% ginger.

The number and percentage occurrence of the isolates from the pito samples are presented in Table 2. *Saccharomyces cerevisiae* was the predominant isolate with the percentage occurrence ranging from 84.75% to 100% in the control pito sample (100% sorghum) and pito produced with 65%: 20%: 15% sorghum: clove: ginger.

Table 2 Number and percentage occurrence of the isolates from the pito samples

Pito samples	Microbial isolates	N	% occurrence
A (295)	*Saccharomyces cerevisiae*	250	84.75
	Candida albicans	40	13.56
	Escherichia coli	2	0.68
	Proteus sp.	1	0.34
	Klebsiella pneumonia	1	0.34
	Enterobacter aerogenes	1	0.34
B (231)	*S. cerevisiae*	220	95.24
	C. albicans	10	4.33
	E. aerogenes	1	0.43
C (10)	*S. cerevisiae*	10	100

The proximate composition (%) of the pito samples are presented in Table 3. There was no significant difference ($p > 0.05$) in the moisture as well as the ash content of the various fortifications with the unfortified control sample. The fat, protein and fiber content of the pito produced with 75%: 15%: 10% sorghum: cloves: ginger, are significantly higher ($p < 0.05$) than that of the control. However, the carbohydrate content of the control sample (100% sorghum) was significantly higher ($p < 0.05$) than the pito samples fortified with cloves and ginger.

Table 3 Proximate composition (%) of the pito samples

Parameters	Sample A	Sample B	Sample C
Moisture	82.64 ± 0.86^{a}	83.55 ± 1.00^{a}	83.27 ± 1.84^{a}
Ash	0.89 ± 0.11^{a}	0.85 ± 0.04^{a}	1.05 ± 0.04^{a}
Fat	4.39 ± 0.06^{b}	5.79 ± 0.03^{c}	4.99 ± 0.35^{b}
Protein	2.10 ± 0.07^{c}	2.80 ± 0.15^{cd}	2.45 ± 0.12^{cd}
Fiber	0.54 ± 0.03^{e}	1.28 ± 0.12^{b}	0.49 ± 0.04^{e}
Carbohydrate	9.42 ± 0.04^{a}	5.73 ± 0.18^{b}	7.75 ± 0.04^{c}

Within the rows, means with different superscripts are significantly different at $p < 0.05$. A = Unfortified pito (100% sorghum), B = 75% sorghum + 15% cloves + 10% ginger, C = 65% sorghum + 20% clove + 15% ginger.

Table 4 presents the bioactive constituents of the pito samples. The tannin, alkaloid, flavonoids and

phenol constituents of the pito samples produced with 75% sorghum + 15% clove + 10% ginger are significantly higher ($p < 0.05$) than that of the control and pito containing 65% sorghum + 20% cloves + 15% ginger.

Table 4 Bioactive constituents (%) of the pito samples

Parameters	Sample A	Sample B	Sample C
Tannin	9.34±0.08^{c}	12.87±0.07^{d}	9.33±0.09^{c}
Alkaloid	8.79±0.06^{a}	22.09±0.02^{b}	14.53±0.33^{c}
Flavonoids	5.80±0.19^{a}	17.64±0.18^{b}	15.45±0.10^{c}
Phenol	24.37±0.43^{e}	28.93±0.14^{f}	25.74±0.22^{g}

Means with different superscripts within the row are significantly different at $p < 0.05$. A = Unfortified pito (100% sorghum), B = 75% sorghum + 15% cloves + 10% ginger, C = 65% sorghum + 20% clove + 15% ginger.

The mean sensory scores of the panelist in terms of colour, flavour, aroma, taste and overall acceptability are presented in Table 5. The pito samples produced with 75% sorghum + 15% cloves + 10% ginger recorded significantly higher ($p < 0.05$) mean sensory scores with respect to colour, flavour, taste and overall acceptability, when compared with the control and the sample produced with 65% sorghum + 20% cloves + 15% ginger. However, the aroma of the pito produced with 75% sorghum + 15% cloves + 10% ginger and 65% sorghum + 20% cloves + 15% ginger are significantly higher ($p < 0.05$) than the control, with no significant difference ($p > 0.05$) between the latter.

Table 5: Sensory scores of the pito samples

Parameters	Sample A	Sample B	Sample C
Colour	5.6±0.23^{a}	6.5±0.06^{b}	5.4±0.21^{a}
Flavour	6.7±0.20^{b}	7.3±0.30^{b}	5.8±0.21^{c}
Aroma	4.1±0.21^{d}	6.9±0.21^{c}	6.3±0.15^{c}
Taste	6.4±0.21^{b}	8.0±0.21^{c}	6.0±0.15^{b}
Overall acceptability	6.8±0.31^{f}	8.0±0.25^{e}	7.0±0.25^{f}

Values with different superscripts within the row are significantly different at $p < 0.05$. A = Unfortified pito (100% sorghum), B = 75% sorghum + 15% cloves + 10% ginger, C = 65% sorghum + 20% clove + 15% ginger.

DISCUSSION

Microbial analysis of the pito samples

The total heterotrophic bacterial, fungal and coliform counts observed in the pito samples (Table 1) were less than 10^4 cfu/ml. This suggests that the pito produced in this study were within the permissible limits of acceptable microbiological standard (Matumba et al. 2011; Centre for Food Safety, 2014). The microorganisms isolated from this study include: *Saccharomyces cerevisiae*, *Candida albicans*, *E. coli*, *Proteus* sp., *Klebsiella pneumonia* and *Enterobacter aerogenes* (Table 2). Similar organisms were isolated from fermented pito and burukutu (Kolawole et al. 2018). Some of these organisms are potential human pathogens and their presence in pito could attract public health attention. The presence of *E. coli*, *Proteus* sp., *K. pneumonia* among others in pito suggests poor handling/unhygienic practices and the use of contaminated water or equipment during pito preparation. However, the absence of most of these bacteria especially in the pito fortified with cloves and ginger suggests the antimicrobial potential of these spices. Olayiwola et al. (2017) reported a very low recovery rate of gram negative bacteria in ogi fortified with ginger. The high number of *S. cerevisiae* obtained in this study may be of interest because the yeast is known to be involved in fermentation (N'guessan et al. 2010; Clavijo et al. 2011), and could be implicated in the fermentation of the beverage. Thus, their presence in pito poses no health risk because they cannot be considered as microbial contaminants.

Proximate composition of the pito samples

Proximate analysis is important because it provides information about the nutritional status of foods and food products, to ensure that consumers are receiving a balanced meal. Pito is a liquid based beverage, thus the very high moisture content (Table 3) recorded in the pito samples is not surprising. In that case, pito should be consumed within 24 to 48 hours of production or stored in an airtight container in a refrigerator. Similar high moisture content was reported in sorghum based beverages; pito and obiolor (Ajiboye et al. 2014). Protein is essential in the body building and repair of body tissues, and is being regarded as one of the key nutritional ingredients in foods (Fennema et al. 2017). The higher amount of protein found in the pito samples fortified with cloves and ginger showed that fortification increased the bioavailability of protein in the beverage when compared to the unfortified pito. Similar higher protein content was reported in 10% and 20% ginger fortified ogi, when compared to the

unfortified control (Olayiwola et al. 2017). Kolawole et al. (2018) reported that whole grain sorghum contains more carbohydrate, proteins and lipids and lesser quantities of fiber, vitamins and minerals. This could be responsible for the low amount of ash and dietary fiber recorded in the pito samples produced in this study. Moreover, the low amount of dietary fiber recorded in this study could also be attributed to the effect of fermentation which hydrolyzed majority of the fibers (pectin, cellulose, hemicellulose and lignin) in the sorghum to simple sugar (Ajiboye et al. 2014). The highest amount of carbohydrate (9.42%) recorded in the unfortified pito could be due to the larger quantity of sorghum (100%) used in the fermentation of the beverage. However, the pito samples fortified with cloves and ginger also recorded appreciable amount of available carbohydrate in the range of 5.73 to 7.75%. This showed that the beverage could serve as a source of energy in form of adenosine triphosphate (ATP). Abimbola and Alagamba (2020) reported a carbohydrate content ranging from 2.75 to 8.3% in pito samples prepared and hawked in Ogun State, Nigeria. Ajiboye et al. (2014) also reported the available carbohydrate content of 5.60 in sorghum based beverage, pito.

Effect of fortification on the bioactive constituent of pito

Bioactive compounds are extranutritional constituents widely distributed in plant based foods, providing health benefits beyond their basic nutritional value. The higher concentrations of these compounds; tannin, alkaloids, flavonoids and phenol in the pito sample produced with 75% sorghum + 15% cloves + 10% ginger (Table 4) may also have contributed to the taste, flavour, aroma and overall acceptability of the sample. However, phenol was found in higher concentration than the other bioactive compounds analyzed. Phenolic compounds are important secondary metabolites with significant physiological benefits for man (Liu et al. 2019). Flavonoids have been reported to act as antibacterials and anti-carcinogens (Huang et al. 1992). Tannin has also been reported to possess a wide range of anti-infective actions and is also useful as anti-tumor agent (Haslam, 1996; Nwoko et al. 2017). The presence of these secondary metabolites in pito further buttressed that the beverage could provide an excellent health benefits to the consumers, especially when fortified with spices such as cloves and ginger.

Effect of fortification on the sensory quality of the produced pito

The higher sensory scores with respect to colour, flavour, aroma, taste and overall acceptability, recorded in the pito sample fermented with 75% sorghum + 15% clove + 10% ginger, compared with the other samples, showed consumer preference for the fortified pito. This implied that fortification of sorghum with 15% cloves and 10% ginger could be considered when preparing pito, as it is the most desirable by the panelists. The reason for the preference of the pito fortified with 15% cloves and 10% ginger could be due to the warm and comforting flavour and taste provided by the spices. The taste of cloves can be described as slightly sweet and spicy, with a hint of bitterness, and when used in combination with cinnamon, nutmeg or ginger, it creates a warm and comforting flavour profile. The unattractive sensory attributes recorded in the pito sample produced with 65% sorghum + 20% cloves + 15% ginger may be attributed to the larger quantity of cloves used, which imparted a bitter taste to the beverage. However, the aroma of the pito samples fortified with ginger and cloves (Table 5) was highly preferred by the panelists, when compared with the control without fortification. The possible reason for the rejection of the unfortified pito with respect to aroma, could be attributed to the off-flavour caused by the presence of microbial contaminants, which alter the quality of the beverage (Rodrigues et al. 2011; Ayirezang et al. 2016). In addition, the preference of the pito sample fortified with 15% cloves and 10% ginger, may be due to the higher concentrations of bioactive compounds such as tannis, alkaloids, flavonoids and phenol recorded in the sample. This could have contributed to the taste, aroma, flavour and overall acceptability of the beverage, thus increasing the nutritional, medicinal and food value (Nwoko et al. 2017) of the beverage.

CONCLUSION

Fortifying pito with cloves and ginger helped to reduce the growth and proliferation of microbial contaminants and pathogens in the beverage. This made the beverage safe for human consumption. Moreover, producing pito with 75% sorghum + 15% cloves + 10% ginger improved the microbial quality, bioactive constituents, nutritional and sensory quality of the beverage, and could provide an excellent health benefits to the consumers. In general, fortifying pito with cloves and ginger at the

right proportion will aid in meeting the microbiological quality, nutritional, medicinal and health needs of consumers. It is therefore recommended that pito should be fortified with cloves and ginger during preparation to reduce the microbial contaminants as the beverage is mostly perpared by rural dwellers where access to clean water is non existence. This will invariably improve the nutritional quality and the health status of the consumers.

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