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Dear Noah,

ACCEPTANCE OF ABSTRACT FOR POSTER PRESENTATION

On behalf of the Conference Planning Committee of the 15th University of Lagos Annual Research Conference, I am glad to inform you that your abstract Ref. No. 20C/SCI/004P, titled

Microbial and Sensory Quality of Pito Beverage Treated With Moringa Seed and Ginger Extract

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has been provisionally accepted for **poster presentation** at the forthcoming conference holding September 22 and 23, 2020. Please be informed that you are to submit your presentation to unilagconf@unilag.edu.ng no later than two (2) weeks from the receipt of this letter.

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- Title page — Slide 1
- Introduction — Slide 2
- Methodology — Slide 3
- Results/Findings — Slide 4
- Conclusion — Slide 5

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We look forward to receiving your full-length paper(s) and your participation at the virtual conference. Further details of the conference will be disseminated on the conference website and WhatsApp group.

Please accept the assurances of our best regards.

Sincerely,

Mrs F.M Odunubi
Secretary

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ABSTRACT

Pito is a traditional brewed alcoholic beverage. Its processing is prone to microbial contamination which result to poor shelf life. The present work investigated the microbial analysis of Pito beverage produced from sorghum treated with varying concentration of moringa seed and ginger extract as preservatives. Standard microbiological method was used. The result of microbiological analysis shows that the Total plate count for the first day ranged from 3.0 $x10^2$ to 14.0$x10^2$ cfu/ml and for the 7 days of storage, it ranged from 2.5$x10^2$ to 6.0$x10^2$ cfu/ml while *Staphylococcus* Count ranged from 4.5$x10^2$ to 0.5$x10^2$ cfu/ml on the first day with no growth on the 7 day. The fungi count of the samples was varied from 10.5$x10^2$ to 2.5$x10^2$cfu/ml during the 0 day and 8.0$x10^2$ to 4.0x 10^2 cfu/ml during 7 days storage for sample. Generally, there was reduction in the microbial growth as storage proceeds in the treated samples with the control sample having the highest growth on both the 0 &7days while no Coliform growth on both 0 & 7 days of analysis in all the treated samples. Sensory evaluation depicts that the result of Overall-acceptability varied from 6.60-8.30 with significant difference ($p<0.05$) across all samples. The sample with ratio (90:5:5) was most preferred among the treated samples as the proportion of moringa seed and ginger extracts increases in terms of overall-acceptability. Hence, moringa and ginger have reduced the microbial count thereby improving the shelf life of pito and organoleptic properties thus it inclusion should be encouraged.

Key words: microbial, sensory, quality, pito, beverage.

INTRODUCTION

The brewing and drinking of traditional beverage are intrinsic part of the culture of African people. Among the problems associated with these traditional products, is the poor shelf life (Ayirezang Saba , Amagloh,, Gonu 2016). Pito is a traditionally brewed alcoholic beverage,

produced mainly from grains of guinea corn *Sorghum* and millet (Kolawole ,Kayode and Akinduyo , 2007). Brewing of Pito originally started in the northern part of Ghana, then to Nigeria but its production is now widely spread over the entire country, serving as a lucrative business particularly for the rural folks. It is produced traditionally by malting, mashing, fermentation and maturation of the grains which are steeped in water over night, after which excess water is drained. The grains are then placed in layers and germinated with periodic moistening. Germination continues for four to five days until the plumule attains a length of about 2.5 cm or longer (Duodu, Amartey , Asumadi-sakyi , Adjei , Quashie , Nsiah-Akoto and Ayanu 2012). The malted grains are spread out in the sun to dry for one or two days after which the dried malt is milled into powder. Boiling of the wort is done among other reasons, to denature malt enzymes and enzymes supplements (Fadahunsi, Ogunbanwo and Fawole, 2013).

The spoilage of Pito is mainly as a result of spoilage microorganisms due to poor food manufacturing practices. The spoilage of most beer is by a diverse array of bacteria and wild yeasts with lactic acid Bacteria being the dominant spoilers, responsible for 60 - 90% of the spoilage that occurs in the breweries (Sakmoto and Konning, 2003) microbial spoilage of alcoholic beverages is of critical importance and for this reason, different methods have been adapted to reduce spoilage, among these methods employed are thermal treatment (pasteurization) and chemical treatments (preservatives). The stability of some traditional sorghum beers is known to have improved through pasteurization, filtration and addition of artificial preservatives (Ellis *et al*., 2005; Onaghise and Izuagbe, 2009; Osseyi Tagba , Karou , Ketevi and Lamboni ,2011) also some of these methods can be sophisticated and expensive for the small scale operator to adopt. According to Onaghise and Izuagbe (2005), the shelf life of Pito was improved to last for a period of 4 weeks when pasteurized at 75^{o}C for 30 minutes with the addition of Sorbic and concentration of 5%. The use of sodium Matabisulphile in foods and beverages is found to have adverse effects such as respiratory tract irritation and anaphylactic symptoms which is life threatening. However, Pito can be produced from sorghum and treated with ginger and *Moringa* seed. (*Sorghum bicolor* L) is one of the most underutilized crops in the semi-arid tropics of Asia and Africa. It is the principal source of energy, protein, vitamins and minerals for millions of the poorest in these regions (Elemo and Okafor, 2011). It is the grain of choice in brewing African traditional beers. Sorghum (Sorghum guinea) is readily available and

acceptable in Nigeria (Okoli *et al*., 2010). Ginger (*Zingiber officinale*) belongs to the family of *Zingiberaceae*, it is an underground stem (rhizomes) of a perennial herb. The useful parts of this crop are the rhizomes which are used as spices in food for its aroma and pungency but have been demonstrated to have both antioxidant and antimicrobial activities (Kolapo *et al.,* 2007). *Moringa oleifera* is the most widely cultivated species in the genus *Moringa*, the only genus in the plant family *Moringaceae*. *M. oleifera*, an outstanding source of nutritional components. The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries. *M. oleifera* leaves has been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants (Mahmood,Mugal and Haq 2010). *Moringa* seeds contain significant sources of minerals (calcium, phosphorus, and iron) and vitamins (A, B and C). It is rich in protein but low in fat and carbohydrates (Olushola 2006).

Preventive medicine has immensely been improved by the used of these natural plant antioxidants.

Protection of food from microbial deterioration has been an important concern to individuals and food industries. Consumers in recent years have a high preference for natural preservatives to chemical-based preservatives due to their adverse health effects which had called for natural, safe and effective preservatives by consumers and producers (Orkega-ramirez *et al.*, 2011). Moringa seed in that regard is also found to possess antimicrobial properties. Information by Eilert, Wolters & Nohrstedt, 2001 on the use of Moringa seed in the brewing of Sorghum beer has not received any attention, therefore using Pasteurization and a natural preservation (Moringa Seed) which is easily accessible in Nigeria and in the tropics (Quarcoo, 2008). With the above problem associated with the production of Pito, novel ways are needed to produce Pito of a better shelf life, this instigated the present research. The purpose of this study is to improve the shelf life of Pito by the addition of moringa seed and ginger extract.

MATERIALS AND METHOD

Sample Collection

Sorghum, ginger seeds and moringa seeds were purchased from Sayedero market Ilaro, Ogun State.

Sample Preparation

The sample collected was brought to the processing laboratory at Federal Polytechnic, Ilaro, Ogun State. Sorghum, moringa seed and ginger seed was used. Sorghum was sorted to remove foreign materials such as dirt and stone.

Preparation of moringa seed extract

Matured moringa seed was manually removed from the seed kernels and dried using an oven drier (65^0C for 5 days), the dried seeds were grounded in a clean martex blender. The moringa seed powder was sieved using a sieve of 500 um mesh size to maintain a fine powder. Dried moringa powder of 50g was added to 500ml distilled water in 1litre conical flask and kept for 1 week in a refrigerator (5^0C) with periodical manual shaking. Extract was filtered using a clean sterilized muslin cloth and then boiled for 30 min with continuous stirring. *Moringa oleifera* seed extract was added in different ratios (5ml, 10ml, 15ml, 20ml and 25ml).

Preparation of ginger extract

Two hundred grams of the ginger spice were separately chopped into small pieces with a clean stainless steel knife. The chopped spices were then blended into 100ml de-ionized water with a Kenwood blender having stainless steel blades until smooth pastes were obtained. The pastes was diluted further with 200 ml de-ionized water and filtered using a clean Muslin cloth. The resulting extracts were stored in clean bottles inside a refrigerator (5°C) until needed for use in pito preparation (8±2°C) until used. (Adesokan *et,al* 2013).

Production of Pito

About 700g sorghum grains was sorted in order to remove stones, dirt and other extraneous materials. The clean grains was thoroughly washed and steep in water for 12 hours and the grains was allowed to germinate on a jute bag for three days. The sprouted sorghum grains was dried and milled. Then mixed with water and boil. The wort was allowed to cool. The insoluble components of the mixture settles and it was been filtered off. Slime extracted from crushed okro stem was then be added to aid in the sedimentation of the non-soluble parts of the mash. Then the wort was allowed to ferment by the addition of yeast in cooking pots containing the cooled wort.

Moringa oleifera seed and ginger extract was then added in different ratios 100:0, 95:5:0, 90:5:5, 85:10:5, 80:15:5, 75:20:5.

Microbiological analysis

The various Pito drink samples were serially diluted in sterile distilled water and appropriate dilutions were plated on nutrient agar, potato dextrose agar (PDA), bairdparker agar, MacConkey agar for total viable, fungal and coliform counts, respectively. Agar plates used were incubated at 35°C for 24hrs, while the PDA were incubated at 28°C for 72hrs and were supplemented with streptomycin to inhibit the growth of bacteria (Lynne, 2013).

Sensory Evaluation

Sensory characteristics of the product were assessed by trained and conversant panel of judges from the polytechnic. The samples were assessed for the following; color, taste, after-taste, aroma, consistency and overall acceptability. The samples were arranged randomly and presented to the judges in the same type of clean and transparent cup and each samples were coded in such a way the panelists recorded the sensory characteristics of each sample using a 9-point hedonic scale as described by (Ihekoronye and Ngoddy, 2005).

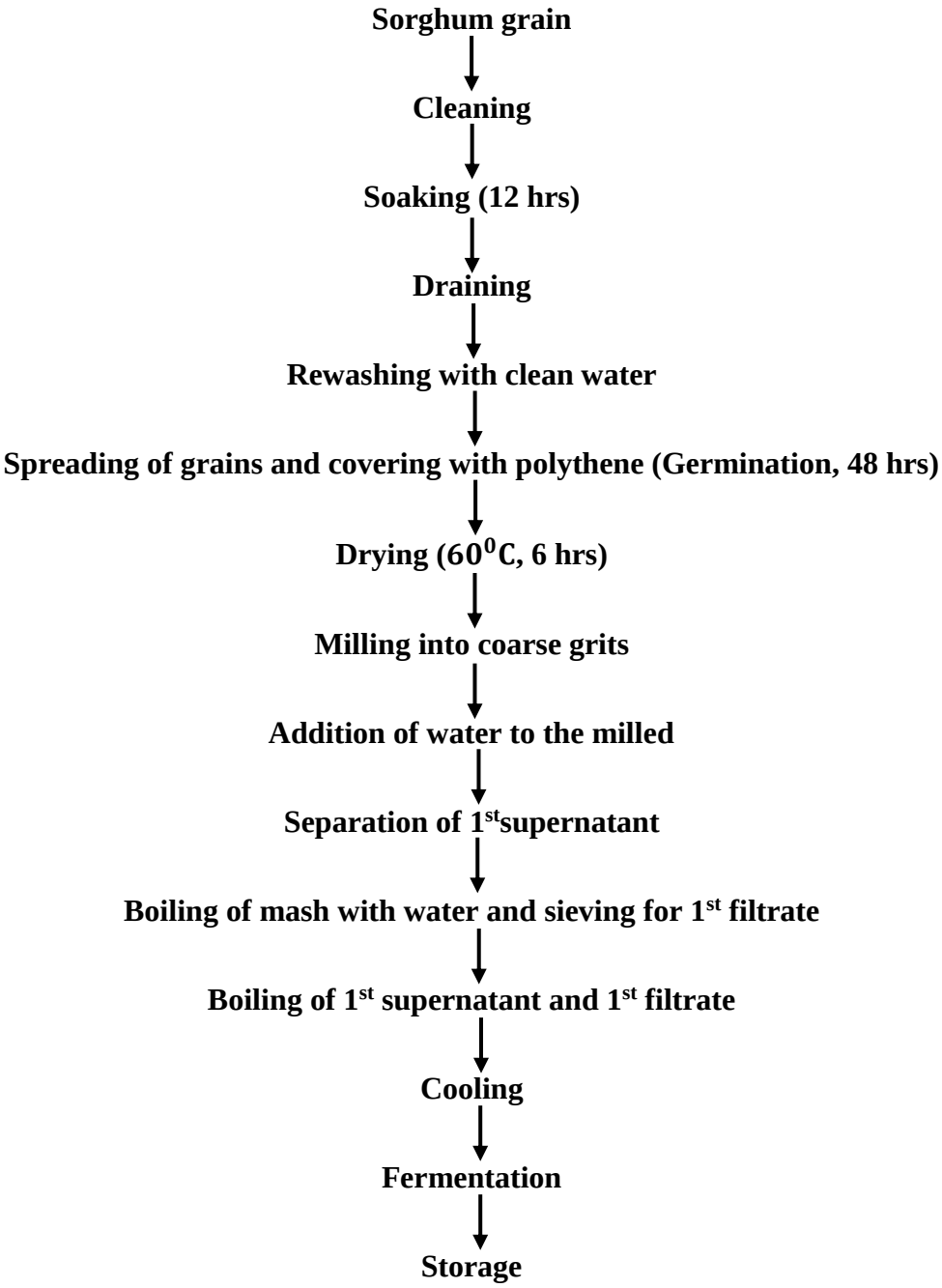

Figure 1. Flowchart for the Production of Pito

RESULTS AND DISCUSSION

The result of the microbial analysis and sensory evaluation of pito treated with moringa seed and ginger extract were shown in Table 1 and 2 below.

Results

Table 1. Microbial count of Pito treated with moringa seed and ginger extract for 0, and 7 days respectively

Samples		Total viable count Cfu/ml x10^2	Fungi Cfu/ml x10^2	Coliform count Cfu/ml x10^2	Staphylococcus count Cfu/ml×10^2
A	**0**	14.00±1.41^{c}	14.50±1.41^{c}	12.00±1.41	6.50±2.12^{c}
	7	6.00±1.41^{b}	8.00±1.41	9.50±0.71^{ab}	3.00±1.41^{a}
B	**0**	8.00±1.41^{b}	9.50±0.71^{ab}	7.00±1.41	4.00±1.41^{bc}
	7	4.50±0.71^{ab}	6.50±0.71	Nil	Nil
C	**0**	6.00±1.41^{ab}	6.00±1.41^{d}	Nil	2.5±0.71^{ab}
	7	4.00±1.41^{ab}	4.00±0.00	Nil	Nil
D	**0**	4.50±0.71^{a}	3.50±0.71^{ab}	Nil	3.00±1.41^{ab}
	7	3.50±0.71^{ab}	Nil	Nil	Nil
E	**0**	3.50±0.71^{a}	3.00±1.41^{a}	Nil	2.00±0.00^{ab}
	7	3.00±1.41^{a}	Nil	Nil	Nil
F	**0**	3.00±1.41^{a}	2.50±0.71^{a}	Nil	0.50±0.71^{a}
	7	2.50±0.71^{a}	Nil	Nil	Nil

***Values are means of duplicate determination ± SD (Standard deviation)*
KEYS: A (100%), B (95:5%), C (90:5:5%), D (85:10:5%), E (80:15:5%), F (75:20:5%).

Table 2. Sensory Evaluation of Pito Treated With Moringa Seeds And Ginger Extract

SAMPLES	COLOUR	AROMA	TASTE	CONSISISTENCY	OVERALL-ACCEPTABILITY
MTH	8.10±0.74^{b}	7.70±0.67^{b}	8.00±0.67^{a}	7.90±0.57^{c}	8.30±0.67^{c}
BPH	7.20±0.42^{ab}	7.71±0.88^{ab}	7.00±1.33^{ab}	7.60±0.84^{bc}	7.40±0.52^{ab}
STP	7.20±0.79^{ab}	7.80±0.79^{b}	8.00±0.82^{b}	7.60±0.52^{bc}	8.10±0.57^{bc}
BCH	6.50±0.85^{a}	7.00±0.67^{ab}	7.10±0.99^{ab}	7.10±0.57^{abc}	7.20±0.92^{a}
STM	6.50±1.18^{a}	6.50±1.27^{a}	6.50±1.08^{a}	6.60±0.97^{a}	6.90±0.99^{a}
FST	6.40±1.65^{a}	6.70±1.25^{a}	6.40±1.17^{a}	6.80±1.23^{ab}	6.60±1.26^{a}

Values are means of duplicate determination ± SD (Standard deviation). Means in the same column with different superscript are significantly different (p>0.05)

KEYS: A: Pito from 100% sorghum (control)
B: Pito from 95% sorghum and 5% ginger extract
C: Pito from 90% sorghum, 5% moringa seed extract and 5% ginger extract
D: Pito from 85% sorghum, 10% moringa seed extract and 5% ginger extract
E: Pito from 80% sorghum, 15% moringa seed extract and 5% ginger extract
F: Pito from 75% sorghum, 20% moringa seed extract and 5% ginger extract

Discussion

Microbial analysis of Pito treated with moringa seeds and ginger extracts

The microbial analysis result of the prepared Pito sample is presented in Table 1 above. The values for Total plate viable counts, Coliform, Fungi and Staphylococcus count during the 0 and 7 days storage after production varied from 2.50-14.00, 7.00-12.00, 2.50-14.50 and 0.50-6.50 x10^2 cfu/ml respectively. The total viable counts ranges from 2.50 to 14.00 x10^2 cfu/g. Sample A had the highest count while Sample E had the lowest microbial counts of 2.5x10^2cfu/ml.

There was general reduction in the growth as storage proceeds. All the treated samples had a lower microbial counts, this is due to the preservative effect of ginger and moringa seed extracts. The Coliform count varied from 7.00 -12.00 $x10^2$ cfu/ml for Sample A (100% Pito) and B (95:5) of moringa seed and ginger extracts used in the production of the Pito, hence contributed to the reduction of Coliforms in the treated samples at 0 day after incubation for 48hrs compared to the high Coliform load in un-treated Pito (control), no Coliform growth was observed at both 0 and 7 day of analysis in the treated samples. The heat treatment from pasteurization of the samples also reduced Coliform in samples prior to storage and this also can be attributed to the hygienic practices adhere to during production which detrimental to some sensitive bacterial (Fadahunsi *et al*., 2013). However, reduction of Coliform load in untreated Pito during storage as observed during storage is as a result of spontaneous microbial activity(accumulation of lactic acid and acetic acid) during storage produced by bacteria , as well as lowering of the pH values in Pito during storage which some Coliform cannot survive (Ray and Bhunia., 2013).

The result for the staphylococcus count of the Pito samples varied from 6.50-0.50 during the 0 day while there was general decreased of growth as the ratio of the moringa seed extract increased, however, there was no growth in the treated samples at the 7 day analysis. The fungi growth of the samples varied from 14.50-2.50 $x10^2$ cfu/ml during the 0 day and 8.00-4.00 $x10^2$ cfu/g during 7 day storage for sample A, B and C. There was general reduction in the growth as storage proceeds, the control sample having the highest growth at both the 0 & 7days (14.50 & 8.00 $x10^2$ cfu/g). There was general decrease in the fungi growth as the proportion of moringa seed extracts increased and this can be attributed to the combination of pasteurization, moringa seed and ginger extract treatment which assisted in the reduction of mould growth to acceptable level, no growth was observed in the D, E and F samples during the 7day analysis. This was in line with previous reports that pasteurization is capable of inactivating microbial activity in traditionally brewed sorghum beers (Ellis *et al*., 2005). The reduction of the growth of fungi in untreated Pito during 7days of storage might have resulted from the exhaustion of nutrients in the products, thus reducing the overall food availability for the microorganisms as reported by other researchers (Fadahunsi *et al*., 2013). Generally, addition of pasteurization, moringa seed extracts and ginger extracts might have been the major contributing factor, influencing the overall reduction of microbial growth in the treated Pito samples, since it has been reported that the souring in

sorghum is owned to the presence of lactic acid bacterial or acetic acid bacterial(Lyumugabe et al.,2012)

Sensory evaluation of Pito treated with moringa seeds and ginger extract

The result for sensory evaluation of the treated Pito is presented in Table 2. The result showed the mean scores for Colour, Aroma, Taste, After Taste, Consistency and Overall-Acceptability varied among the samples from 6.40-8.10, 6.50-7.80, 6.20-7.80, 6.40-8.00, 6.60-7.90 and 6.60-8.30 respectively.

The result of Colour varied from 6.40-8.10 with significant difference ($p<0.05$) across all samples. Sample A (100%) was rated high with mean score of 8.10 while sample F (75:20:5) had the least value. The Aroma varied from 6.50-7.50 with significant difference ($p<0.05$) across all samples. Sample C (90:5:5) having the highest mean score of 7.50 while sample F (75:20:5) had the least mean score value. The result of Taste varied from 6.40-8.00 with significant difference ($p<0.05$) across all samples. Sample A and C having the highest mean score of 8.00 while sample F had the least mean score value. The result of Consistency varied from 6.60-7.90 with significant difference ($p<0.05$) across all samples. Sample A (100) having the highest mean score of 7.90 while sample E (80:15:5) had the least mean score value.

The result of Overall-Acceptability varied from 6.60-8.30 with significant difference ($p<0.05$) across all samples. Sample C (90:5:5) was rated high along with control sample A, Hence, SampleC (90:5:5) is the most preferred among the treated samples in terms of overall-acceptability.

CONCLUSION

The study has shown a decrease in the microbial growth as compared to the un-treated sample in all the attribute rated and thus, extending the shelf life over three weeks. However, the sensory results showed that sample C was preferred among the treated samples alongside with the control by the consumers. Based on the findings of this study, the research acknowledged the improvement on shelf life of Pito through the addition of moringa seed and ginger extract. Although Pito samples containing higher percentage of moringa seed and ginger extract were less preferred by the consumer.

RECOMMENDATION

The incorporation of the moringa seed and ginger extract treatment up to 10% is encouraged. Futher study on physicochemical analysis should also be carry out.

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