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Assessment of the Microbial Status of 'OGI' Subjected to Varying Storage Conditions

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ABSTRACT

Adequate food supply all the year round in most developing Nations has been seriously hampered by contamination, deterioration in storage, and inadequate storage facilities. Moisture, Relative Humidity and Temperature affect food in storage indirectly through their influence on the agents of biological deterioration and directly through physical and chemical processes. The microbial qualities of Ogi kept under three different conditions were assessed at the microbiology laboratory of Achievers University, Owo. The samples assessed were procured from local producers at the local market and taken to the laboratory in sterilized nylon bags for examination. Before analyzing the samples, they were placed under three storage condition viz; under water (Treatment 1 or T_1), in refrigerator (Treatment 2 or T_2) and air-dried into wettable powder (Treatment 3 or T_3). All the samples were covered with aluminum foil to prevent cross contamination during handling. The samples were held under these conditions for 1 week after which subsamples were taken from the original samples for analysis. The subsampling and analysis procedures were replicated three times at weekly intervals. The water in T_1 was changed every 24 hours to prevent the growth of fungi. The three media; Nutrient Agar (NA), Potato Dextrose Agar (PDA) and MacConkey (MA) used for the analysis were prepared according to manufacturer's specification. Twenty eight grams (28g) of Nutrient agar powder was weighed using the weighing balance and was dissolved in 1 litre of sterile distilled water in a conical flask. The mixture was thoroughly mixed and boiled to ensure complete dissolution of the powder. The conical flask was corked with cotton wool and later with aluminum foil and sterilized by autoclaving at 121°C for 15 minutes. The same procedure of preparation was used for the preparation of Potato Dextrose Agar (PDA) and MacConkey Agar (MA) with thirty nine grams (39g) and fifty two grams (52g) of powdered media respectively. Identification and characterization of bacteria isolates was done based on morphology, staining reactions and biochemical tests. Fungi identification was based on morphology and

microscopic examinations. The results of the research indicated that storage conditions exert a great deal of effect on the microbial qualities of Ogi. The bacterial isolates identified in the three samples include *E. coli, Lactobacillus sp, Staphylococcus sp, Flavobacterium sp and Vitrobacterium sp.* The results shows that Ogi samples under water (T_1) has the highest number of isolates followed by refrigerated Ogi sample (T_2) and dried Ogi sample (T_3) in that order. The trend indicates that moisture and temperature are important factors required by microorganisms to thrive in stored food commodities.

Keywords: Microbial, Assessment, Maize, Ogi, Storage

INTRODUCTION

Ogi is a fermented cereal porridge from West Africa, typically made from maize (*Zea mays*), Sorghum (*Sorghum vulgare*), or Millet (*Triticum sativum*), (FAO, 2006). Traditionally, Ogi is produced by soaking the grains in water for 2 to 3 days before wet milling and sieving to remove the husks. The filtered cereal is then allowed to settle and to ferment for up to three days until sour. In Africa, Ogi is of great importance in many aspects. According to available literature, West African mothers breastfeed their babies for 12 months, many urban and rural women breastfeed up to 18 to 24 months (Kazimi and Kasini, 1979; Amar, 1989). These reports indicate that there is early supplementation with solid foods or early weaning, although the majority of women start weaning their infants at age 3 to 4 months of life. In Nigeria, the usual first weaning food is Ogi called either "pap", "akamu" or "koko" (Osunbor, 1980; Cherian, 1981; Longhurst, 1984).

Many research studies have been carried out on the microbiological analysis of Ogi in several parts of Nigeria. Ezendianefo and Dimejesi (2014) determined the microbial quality of Ogi in Nnewi markets in Anambra State. The results show that total bacterial counts ranged from 3.0×10^8 to 7.5×10^8 cfu/g. Afolayan, et al (2009), investigated into sorghum based ogi (ogi baba) storage characteristics. The experiment aimed at checking the influence of accelerated drying on the quality and storage life span of the product. The performance was accessed using some organoleptic (odour, colour and fungi invasion count)

characteristics at a weekly interval for four weeks by 10 human subjects confirmed medically fit in their use of olfactory and visual organs. The experimental samples were opened to the atmosphere while the control is sealed immediately after drying the product and was cooled to room temperature. The 60 minutes heating was found to have the most stable colour and odour and this property is even more stabilized when the product is sealed (as in the control sample). On the other hand, the 60 minutes sample is the most loaded with fungi as the experiment progresses but no fungi infestation when sealed, the 20 and 40 minutes samples (even when sealed) had fungi infestation.

Mbakwem and Udembga (2012) examined the microbiological quality of untreated and salt – treated ogi kept at room temperature (28°C) for three weeks. The bacteria identified were *Lactobacillus sp., Corybacterium sp., Enterobacter sp., Citrobacter sp., Micrococcus sp., Staphylococcus sp.,* while the fungi species identified include *Aspergillus sp., Rhizopus sp., Fusarium sp., and Saccharomyces sp.* Both treated and untreated contained bacterial and fungi isolates with microbial load decreasing with increase salt concentration. The work revealed that salt can serve as an inexpensive material for extending the shelf – life of ogi. Okwute and Olafijadi (2013) determined the effects of ginger (*Zingiber officinale*) on microbial load of ogi. The results revealed that samples containing no ginger had the highest microbial count of 7.3 x 10^5 cfu/g, while microbial count reduces with increased level of ginger treatment. While most of the past research efforts centers on the improvement of the organoleptic status and improvement of the shelf-life of of ogi, the objective of this research effort is centered on studying the effect of storage on microbial quality of ogi.

Storage Techniques

Most of the present day techniques used in food storage were known and practiced thousands of years ago. Some of the evidences available are sufficient to indicate that the modern storage techniques of drying

(both natural and artificial), sealing(airtight storage),cooling, freezing, fermentation, picking, salting and smoking, were all used, though crudely, long time ago. The principal weapon in storage practices relates to control of storage environment or handling conditions. Control over temperature is the most significant environmental factor as the rate of deterioration from all causes is affected by temperature. The rate of normal metabolism decreases with decreasing temperature, but effects on commodity quality are more pronounced at the lower end of temperature scale, a change of 2°C and 20°C has less effect on metabolism than the same temperature change at 3°C. It would seem that the ideal storage temperature is just above freezing point where metabolism is slowest for raw commodities. However, the onset of abnormal metabolism at reduced temperature becomes a limiting factor for many commodities. The beneficial effect of reduced temperature on microbial growth is an important consideration in storage systems.

Problems of Stored Foods

Adequate food supply all the year round in most developing Nations has been seriously hampered by contamination, deterioration in storage and inadequate storage facilities. Moisture, Relative humidity and temperature affect food in storage indirectly through their influence on the the agents of biological deterioration and directly through physical and chemical processes.

Biological Deterioration of Stored Food Commodities

It has been observed that the organisms that cause deterioration of stored food commodities require suitable moisture content and a favourable temperature to thrive and multiply. The maintenance of cool and dry conditions in and around stored food commodities therefore, will reduce microbial spoilage and contamination.

Importance of Effective Storage:

Effective storage ensures that:

1. Food is available all the year round at affordable prices.
2. The food consumed by the populace is of good quality and as such will lead to improvement in health of the people.
3. Producers are encouraged to increase their production and also take advantage of reasonable increase in prices at off season, thereby making more profit.
4. There is a reduction in the usual wastage, and losses of food commodities produced.
5. There is a boost in the export sector as excess raw food products available for the local industries may be exported after satisfying the local needs.
6. Improved quality of stored commodities will boost the goodwill of producers and exporters.

MATERIALS AND METHODS

Collection of Sample

The samples assessed were procured from local producers at the local market and taken to the laboratory in sterilized nylon bags for examination. Before analyzing the samples, they were placed under three storage conditions; under water (Treatment 1 or T_1), in refrigerator (Treatment 2 or T_2) and air-dried into wettable powder (Treatment 3 or T_3). All the samples were covered with aluminum foil to prevent cross contamination during handling.

Preparation of Media

The three media i.e. Nutrient Agar (NA), Potato Dextrose Agar (PDA) and MacConkey (MA) used for the analysis were prepared according to manufacturer's specification. Twenty eight grams (28g) of Nutrient agar powder was weighed using the weighing balance and was dissolved in 1L of sterile distilled water in a conical flask. The mixture was thoroughly mixed and boiled to ensure complete dissolution of the powder. The conical flask was corked with cotton wool and later with aluminum foil and sterilized by autoclaving at 121°C for 15 minutes. The

same procedure of preparation was used for the preparation of Potato Dextrose Agar (PDA) and MacConkey Agar (MA) with thirty nine grams (39g) and fifty two grams (52g) of powdered media respectively

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF STORED OGI

Hydrogen ion Concentration (pH)

The $_{p}H$ of ogi samples were determined using $_{p}H$ meter ($_{p}H$ – 3c) meter. 5g of each sample was macerated and transferred into 100ml of distilled water in a conical flask. The mixture was agitated to obtain thoroughly homogenous sample. The $_{p}H$ meter was standardized with buffer solution of $_{p}H$ 4.0, 7.0 and 9.0 according to user's manual. The electrode was inserted into the ogi sample solution to determine the $_{p}H$ of samples. The procedure was carried out three times for each sample and the mean $_{p}H$ of sample was calculated.

Moisture Content

The moisture content of the samples was determined using the AOAC standard method; 5g of samples were weighed into a previously weighed crucible. This was dried in an oven at 170°C for 1hr. The samples were allowed to cool down in a desiccator in which silica gel was used as the desiccant. Subsequent weighing was done at 30 minutes interval for three (3) consecutive readings until a constant weight was obtained. The percentage moisture content was then calculated with the following formula:

$$\% \text{ Moisture content} = \frac{W_2 - W_1}{W_1 - W_0} \times 100$$

Where:

W_0 = Weight of empty crucible

W_1 = Weight of crucible + Weight of 5g of fresh ogi sample

W_2 = Weight of crucible + weight of dried ogi sample.

Temperature

The temperature of ogi samples were assessed using a liquid expansion thermometer. Measurement procedures were carried out at the beginning of each analytical procedure and the mean temperature for each sample was determined.

Texture

For simple comparison of samples, texture was qualitatively assessed by observation and feel.

MICROBIOLOGICAL ANALYSIS

Serial Dilution Preparation

The tenfold serial dilution preparation for the microbiological analysis of ogi samples was done using ten (10) sterilized test tubes labelled 10^1 to 10^{10}. 1gm.of the sample was aseptically weighed and introduced into a sterile mortar and pestle. The ogi sample was macerated and introduced into a test tube containing 9ml of sterile distilled water. The mixture was thoroughly mixed to homogenize and this gave 10^1 dilutions. 1ml of the mixture was pipetted and transferred into another test tube containing 9ml of sterile distilled water. The mixture was properly shaken and this gave 10^2 dilutions. The procedure was repeated sequentially until the 10^{10} dilutions was reached. The 10^5 dilutions were chosen for analysis.

Total Bacteria Count

Isolation and enumeration of organisms was done using pour plate method. 1 ml of the aliquot from 10^5 dilution was pipetted aseptically into two separate sterile petri dishes, cooled nutrient agar was poured aseptically and the plates swirled to mix the inoculums with the medium. The plates were allowed to set and inoculated at 37°C for 24hrs. The plates were examined for growth and the colonies counted after 24hrs of incubation using colony counter.

Total Enteric Count

The procedure used for the total enteric count was the same as those described under total bacteria count except that the media used for isolation and enumeration of enteric organisms was MacConkey agar.

Total Fungal Count

The isolation and enumeration of fungi was done using pour plate method. 1ml of the aliquot from 10^5 was pipetted aseptically into two separate sterile petri dishes. Cooled molten Potato Dextrose Agar (PDA) was poured aseptically and the plates were gently swirled to mix the inoculums with the medium. The plates were allowed to set and inoculated at 25°C for 72hrs. The plates were observed for growth and colony counting every 24 hr. after inoculation. Plates devoid of any visible growth are discarded after 72 hr. of inoculation.

IDENTIFICATION AND CHARACTERIZATION PROCEDURES

Morphological Characteristics

Morphological characteristics of bacterial colonies were done based on the criteria of Berger's Manual of Determinative Bacteriology. These include the shape of colonies, elevation, optical characteristics, consistency and pigmentation. Fungi were described based on their shape, spore, color or pigmentation and hyphal development.

Biochemical Tests

The biochemical tests carried out for identification of isolates to the species level include Catalase test, Citrate test, Indole test, Methyl red test and Sugar fermentation test.

RESULTS AND DISCUSSIONS

Results of the physicochemical and microbial assessment of stored ogi indicated that storage conditions exert a great deal of influence on the quality of the commodity. The results obtained on the various aspects of

the physical, chemical and microbial properties examined are presented on Table 1.

Table 1: Physicochemical and Microbial Qualities of OGI Samples Subjected to various methods of Storage*

PARAMETERS	WET OGI SAMPLE	DRIED OGI SAMPLE	FROZEN OGI SAMPLE
Hydrogen ion concentration ($_pH$) Moisture Content (MC) % Taste. Texture Temperature	6.65 75 Sweat Smooth 28°C	5.60 40 Sour Gritty 32°C	6.50 60 Sweat Hard 0°C
Total Bacteria Count (Cfu/g)	a. 5.5×10^5 b. 5.3×10^5 c. 5.1×10^5	a 3.5×10^5 b 3.0×10^5 c. 3.2×10^5	a 4.0×10^5*** b 5.0×10^5 c. 3.0×10^5
Total Enteric Count (Cfu/g)	a 5.1×10^5 b 5.3×10^5 c 5.3×10^5	a 3.5×10^5 b 3.0×10^5 c. 2.0×10^5	a 4.0×10^5 b 2.0×10^5 c. 5.0×10^5
Total Fungal Count (Cfu/g)	a 0.0×10^5 b 0.2×10^5 c 0.1×10^5	a 1.0×10^5 b 1.3×10^5 c. 1.0×10^5	a 1.3×10^5 b 1.1×10^5 c. 1.2×10^5

*Values mean of three replications **T_1 = Wet ogi T_2 = Dried ogi T_3 = Frozen ogi *** Values records of three replications sample^{-1}

PHYSICOCHEMICAL PROPERTIES

It is evident from the above results that storage condition has effect on the physicochemical and microbial properties of ogi. The results show correlation between storage conditions and physicochemical properties of stored products. Elsewhere, it has been observed that most fungal and bacterial pathogens require free water for spore germination, consequently, infection is favored by prolong warm, wet periods with high relative humidity. From the above results, two inferences that can

be drawn are in relation to the pH of the samples and their taste. The pH in both the wet and frozen samples are close to neutrality, but pH in the dried sample is slightly acidic. This situation might be responsible for the sour taste noticed in the dried ogi sample. Also, texture of ogi is affected by method of storage as shown in the above results. While the wet ogi tends to retain it smooth texture throughout the period of experimentation, the dried sample assumed a gritty texture with the frozen ogi becoming hard. These results are in consonance with the findings of Adeyemi (1983) and Nnam, (2000) who reported that textural and rheological qualities are affected by factors such as cereal type, milling techniques, fermentation process, particle size of ogi, and fortification, storage and handling of ogi may as well exert some influence on these properties as shown by the results obtained in this research (Table1).

MICROBIAL COUNTS

The mean microbial counts of ogi samples subjected to various storage conditions are shown in Table 2. From the results it is evident that all the samples contain microorganisms.

However, the level of microbial load varies within the samples. Microbial loads in sample T_1 i.e. wet ogi sample under water ranges between 5.1×10^5 - 5.5×10^5 Cfu/g (bacterial), $5.1 \times 10^5 - 5.3 \times 10^5$ Cfu/g (enteric) and $0.0 \times 10^5 - 0.2 \times 10^5$ Cfu/g (fungal). Microbial loads in T_2 i.e. dried ogi samples are 3.0×10^5 -3.5×10^5 Cfu/g (bacteria), $2.0 \times 10^5 - 3.5 \times 10^5$ Cfu/g (enteric) and $1.3 \times 10^5 - 1.0 \times 10^5$ Cfu/g (fungi), while microbial loads in T_3 i.e. frozen ogi are $3.0 \times 10^5 - 3.5 \times 10^5$ Cfu/g (bacteria), $2.0 \times 10^5 - 5.0 \times 10^5$ Cfu/g (enteric), and $1.1 \times 10^5 - 1.3 \times 10^5$ Cfu/g (fungi). These results shows that significant differences exist in the levels of microbial load of stored ogi, in relation to the storage method used (Table 1). For instance wet ogi sample under water (T_1) has a fungal load of (5.5×10^5 - 5.1×10^5 Cfu/g) which is higher than those of dried sample (T_2) ($3.5 \times 105 - 2.0 \times 10^5$ Cfu/g) and frozen sample (T_3) ($5.0 \times 10^5 - 2.0 \times 10^5$ cfu/g)

respectively. The results also show that bacteria counts are lower in dried ogi samples. This is an indication that moisture levels and temperature ranges play significant roles in the level of microorganisms present in stored food commodities as higher moisture contents and optimum temperature have been identified as factors that favour the multiplication and growth of microbes. Adeyemi (2000) reported that the principal weapon in storage practices relates to control of the storage environment or handling conditions, control over temperature is the most significant environmental factor as the rate of damage from all causes is affected by temperature and another important environmental condition include the concentration of water vapour around stored products. The lower enteric and bacteria counts for dried ogi samples when compared to wet and frozen ogi samples seems to support these assertions. (Table1).

Research has also shown that the presence of moisture in food items promotes microbial deterioration and invasion of food items by organisms, therefore, the lower the water content, the lower the microbial load, hence the lower the level of deterioration. Fungal counts results for this experiment seems to support these assertions as there seems to be no significant difference in fungal counts for all samples. (Table1). However variations exist in the type of bacterial isolates identified in samples as shown in Table 2 below.

Table 2: Distribution of Bacterial and Fungal Isolates in OGI Samples subjected to various Methods of storage.*

BACTERIAL ISOLATES	T_1	T_2	T_3**
Proteus sp.	_	_	_
Escherichia coli	+	+	+
Lactobacillus sp.	+	_	+
Staphylococcus sp.	_	+	+
Flaviobacteria sp.	+	+	_
Vitrobacterium sp	_	+	+
FUNGAL ISOLATES			
Aspergillus sp	+	+	_
Rhizopus sp	_	+	_
Fusarium sp	_	_	_
Saccharomyces sp.	+	+	+

KEY

- = Not Present + = Present *Values record of three replications **

T_1= Wet ogi T2 = Dried ogi T_3= Frozen ogi

The various types of bacterial isolates indicated in this research include *Escherichia coli, Lactobacillus sp., Staphylococcus sp., Flaviobacteria sp and Vitrobacterium sp.* While fungi isolates include *Apergillus sp, Rhizopus sp, Fusarium sp and Saccharomyces sp*. From the results it is clear that no sample is devoid of microorganisms. This is an indication that storage methods may not necessarily be the only way of preventing contamination of food items. While they may reduce the level of microbial contamination, the combination of various interception and food safety methods might be essential.

CONCLUSIONS

The results of the research indicated that storage conditions exert a great deal of effect on the microbial qualities of Ogi. The bacterial

isolates identified in the three samples include *E. coli, Lactobacillus sp, Staphylococcus sp, Flavobacterium sp and Vitrobacterium sp.* The results shows that Ogi samples under water (T_1) has the highest number of isolates followed by refrigerated Ogi sample (T_2) and dried Ogi sample (T_3) in that order. The trends indicate that moisture and temperature are important factors required by microorganisms to thrive in stored food commodities. In the present circumstances, storing Ogi in dried form seems to be the safest method.

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