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Microbial Quality and Sensory Properties of Ogiri Produced from Watermelon (*Citrullus lanatus*) Seed

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Abstract

The aim is to study the microorganisms involved in fermentation, their occurrence and the organoleptic properties of fermented watermelon (*Citrullus lanatus*) seeds. The watermelon seeds were dehulled, sorted, washed and boiled for 4 hours and fermented naturally for nine days. Microorganisms isolated from the fermenting watermelon seeds were *Bacillus sp*, *Lactococcus sp*, *Pediococcus sp*, *Streptococcus sp*, *Micrococcus sp* and *Alcaligenes ps* for bacterial, while the fungi obtained were *Mucor sp*, *Rhizopus sp*, *Fusarium sp*, *Candida sp* and *Saccharomyces sp*. The bacterial count increased from 4 x10^1 cfu/mL to 1.21 x 10^{10} cfu/mL while fungal count also increased from 8 x 10^1sfu/mL to 1.2 x 10^9sfu/mL during the period of fermentation. The pH and temperature increased from 6.3 to 8.7 and 29°C to 40°C respectively. The total titratable acidity decreased from 0.176% to 0.027%. The mean sensory characteristics value of fermented sample as scored by the panelist shows that the texture of the fermenting sample become softer as fermentation progressed. The aroma became more pungent with a strong ammonia odour responsible for the characteristic flavour and a darker colour of product. The overall acceptability of fermented sample was adjudged like very much while the sample bought from the market was adjudged like moderately.

Keywords: Fermentation, Microorganisms, Temperature, Sensory, Panelist, Acceptability, Organoleptic, Titratable acidity.

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Contents

Contribution of this paper to the literature

This study contributes to existing literature by revealing that watermelon seed an unconventional substrate was fermented. Organisms involved were microbiota from harvest and post-harvest environment, and they played a substantial role in the fermentation process. Which resulted to the biochemical transformation which altered the appearance, texture and flavor of fermented watermelon seeds given it an overall acceptability adjudged like very much. Which supports that fermented watermelon seeds could be additives in animal and baby weaning feed.

1. Introduction

Ogiri is a fermented product of African oil bean. In Nigeria castor oil, melon, fluted pumpkin, African yam and Bambara seeds are used for the production of Ogiri. Such class of fermented product are known as condiment. Condiment are fermented products usually produced from fermented vegetable proteins, they are craft-based [1]. The methods employed in the production of fermented condiments differ from one community to another in many areas. In Nigeria, they are made in traditional ways, observance of good manufacturing practices and control of environmental conditions during the production phase gives a good product, consistency in product quality and stability is difficult to maintain because the fermentation is spontaneous and not controlled using starter culture [2-8]. The substrates used for the production of condiment are diverse as regions are diverse and more than one raw material can be used to produce condiments. Most of the seeds used for condiment production are inedible in their raw, unfermented or cooked state [1]. Watermelon (*Citrullus lanatus)* is a horticultural crop known for its juicy sweet fruit [9-11]. It originated from Namibia but has been domesticated in almost all parts of the world especially warmer climates [12]. It is an important but underutilized crop. The seeds are considered waste but research has reported it as a highly nutritive seed with large amounts of proteins and beneficial minerals [13]. Watermelon seeds are one of the main underutilized fruits grown in warmer parts of the world [12]. Therefore, knowledge on the nutritive and anti-nutritive content of watermelon seed will encourage their consumption in different ways and the re-utilization of the large quantity of seeds thrown away as waste even with their hidden nutrients [12]. Presently, there are limited literatures on the microbiological effect on the nutritional properties. In this study, the microbial quality and sensory properties of fermented seeds of watermelon used to produce a condiment was evaluated.

2. Materials and Methods

2.1. Sampling Sites

Matured, fresh, healthy watermelon fruits were bought from Sasa market in Akure, Ondo State Nigeria. The fruits were cut open with a sterile knife, the seeds were collected then washed in distilled water until clean, dried then dehulled and kept in airtight container until used.

2.2. Sample Collection and Confirmation

The sample was taken to the horticulturist in the Department of Plant Science and Biotechnology, Adekunle Ajasin Universiy, Akungba Akoko, Ondo State, Nigeria where the seeds were confirmed as watermelon (*Citrullus lanatus)* seeds.

2.3. Sample Preparation and Microbiological Analysis

The prepared water melon seeds (600 g) were pressure cooked in the autoclave for 4 hours inside sterilized aluminum pot, excess water was drained off. A sterilized spatula made of stainless steel was used to take part of the boiled, watermelon seeds in to the fermenter. The boiled watermelon seeds were wrapped with jute bags put into calabash and covered with sterile cocoa leaves for nine days.

2.4. Isolation and Characterization of Organisms Involved

One gram (1g) fermenting water melon seeds were aseptically collected at 24 hours intervals for nine days, grind into paste with a sterile mortar and pestle and was aseptically transferred into the stock solution, a test tube containing 9.0 mL sterile distilled water labelled as 10^{-1}. The dilution process was carried out serially until it got to the test tube labelled as 10^{-9}. Experiment was in triplicate. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used for the isolation of bacteria and fungi respectively. The 10^{-3} to 10^{-9} of serially diluted sample above was used. In each diluted sample, 0.5 mL was transferred to the center of a sterile Petri dish. About 20 mL of molten NA and PDA agar cooled to 45°C were then added to each plate respectively and rocked gently to facilitate mixing the agar with the sample. The NA plates were placed at 37 ± 2°C for 24 hours, while PDA plates were kept at 25 ± 2°C for 48-72 hr. The distinct bacterial colonies that grew on the NA were examined physically for their morphological characteristics such as colour, shape, size, elevation, surface and edges. They were then manually counted. Fungi were also observed for their morphological characteristics such as pigmentation during growth, hyphae form, conidia form, growth rate and diameter size of each colony was measured using a meter rule during growth on the PDA plates. Colony counting was carried out visually by counting the number of visible colonies that appeared on the plates. Calculation of colony forming unit (CFU) per gram for the bacteria and the spore forming unit (SFU) per gram for the fungi was based on the formula:

$$\text{CFU/gram or SFU/gram} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{ml of sample suspension}}$$

2.5. Physico-Chemical Analysis

2.5.1. Determination of pH

Fermenting watermelon seed (2g) was weighed and grind into smooth paste then homogenized with 20mL of sterile distilled water in a 50 mL beaker. The pH electrode was immersed into the solution and the reading was noted. This was done for nine days. A pH 211 microprocessor, Hanna instruments.

2.6. Temperature and Total Titratable Acidity (TTA)

The temperature was taken by inserting a sterile Thermometer into the batch of fermenting watermelon seeds while standard method of AOAC [14] was used for TTA.

2.7. Sensory Evaluation

Twenty panelists were selected which consist of venders, producer and individuals. These panelists were used to evaluate the ogiri produced from watermelon seeds for organoleptic characteristics using the nine-point hedonic scale.

3. Results

Table 1. Microbial counts of watermelon seeds fermented for the production of a condiment.

DAYS	CFU/ML	SFU/ML
1	4 X10^1	8 X10^1
2	3.6 X10^4	5.2 X10^3
3	7 X 10^7	7.3 X 10^6
4	1.48 X10^9	1.68 X 10^7
5	1.82 X 10^9	1.82 X 10^8
6	1.78 X 10^{11}	1.98 X 10^9
7	1.74 X 10^{11}	1.72 X 10^9
8	1.56 X 10^{11}	1.41 X 10^9
9	1.21 X 10^{11}	1.20 X 10^9

4. Results and Discussion

Result of the total viable count during fermentation of watermelon seeds as shown in Table 1 reflects that the organisms were in their exponential phase of growth [15] and they increased all through the fermentation period which shows that the fermenting watermelon seed was a suitable substrate for microbial growth.

Table 2a. Cultural, morphological and biochemical characteristics of bacterial isolates obtained during fermentation of watermelon to produce condiment.

S/no	Gram Staining	Cell Shape	Motility	spores	Indol production	Citrate Utilization	Nitrate reduction	Methyl red	Voges proskauer	Oxidase test	Catalase test	glucose	maltose	manitol	fructose	sucrose	lactose	sorbitol	Tentative identity
IS1	-	Rod	+	-	-	+	-	-	-	+	+	+	+	-	-	+	-	-	Alcaligenes sp
IS2	+	Rod	+	+	-	+	+	-	-	-	+	+	+	+	-	+	-	-	Bacillus subtilis
IS3	+	Rod	+	+	-	+	+	-	-	-	+	+	+	-	-	+	-	-	Bacillus megaterium
IS4	+	Rod	+	+	-	+	+	-	-	-	+	+	+	-	-	+	-	-	Bacillus pumilis
IS5	+	Rod	+	+	-	+	+	-	-	-	+	+	+	-	-	-	-	-	Bacillus firmus
IS6	+	Cocci	-	-	-	+	+	-	-	+	+	+	+	-	-	+	-	-	Micro coccus sp
IS7	+	Cocci	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+	+	Streptococcus sp
IS7	+	Rod	-	-	-	+	-	-	-	+	+	+	+	-	-	+	-	-	Lactococcus sp
IS9	-	Rod	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	Pseudomonas sp
IS10	+	Cocci	-	-	-	+	+	-	-	-	-	+	+	-	-	+	-	-	Pediococcus sp

Note: Key: + Positive, – Negative.

Table 2b. Cultural and morphological characteristics of fungal isolates, obtained from watermelon seeds fermented to produce condiment.

S/N	Growth Rate	Diameter of Colony After 7days on PDA	Pigmentation During Growth	Hyphae Form	Conidium Form	Name of Organism
1	Fastidious	At 25°C on PDA 8CM 3days	White to yellow tater dark grey black sporangia	Upright phialide	Branched out from the phialide	Mucor hiemalis
2	Fastidious	8cm in 4days	White to brownish	Upright phialide	Branched out from the phialide	*Rhizopus stolonifer*
3	Moderate	7cm	White to pink	Short, broadly ending non-proliferating phialides	Conidiophores richly branched but not in spirodochia usually one celled macro-conidia	*Fusarium verticilliodes*
4	Moderate	4cm	Creamy	Pseudohypha formed	Ovid shape produced laterally mycelium	*Candida krusei*
5	Moderate	5cm	Creamy	Pseudohypha formed	Ovid shape produced laterally mycelium	Saccharomyces sp
6	Moderate	4cm	Creamy	Pseudohypha formed	Ovid shape produced laterally mycelium	*Candida tropicalis*

At day one, the total viable bacterial count was 4.0 x 10^1 cfu/g and increased to 1.21 x 10^{10} cfu/g on the ninth day. Fungal count was 8 X10^1 sfu/g it increased to 1.20 X 10^9 sfu/g Table 1. The cultural and morphological characteristic showed a continuous and consistent succession of microorganisms as reflected in Table 2(a and b). The most predominant bacteria were *Bacillus* species, and *Alcaligene*s sp while for fungi *Sacharomyces cerevisae*, C*andida* sp, and *Mucor* sp. were the most predominant. Table 3. The presences of some organisms maybe due to contamination or microbiota from harvest or post-harvest environment. From the list of organisms isolated, only few of them can be considered to play a substantial role in the fermentation process. *Bacillus* species were prominent from the start to the end of the fermentation process, *Bacillus* species can grow on ammonia or nitrogen source during melon fermentation to produce condiment [16, 17]. The combination of *Bacillus* species, and *Alcaligenes* species during fermentation of melon condiment are capable of producing the quality characteristic of good fermented melon condiment [11]. Result of physico-chemical parameters is shown in Table 4. Temperature increased from 29°C to 40°C. This may be due to increase in cell population and the metabolic activities going on within the fermenting condiment. The pH increased from 6.3 to 8.7. This could be due to the high protein content of the fermented melon which was hydrolysed to amino acid and ammonia by the dominant microorganisms as reported by Odunfa [2]; Sanni, et al. [18]. The total titratable acid (TTA) decreased from 0.127% to 0.027%. The observed increase in pH and decrease in titratable acidity of the fermented food could be due to presences of some lactic acid bacteria which degrades carbohydrates Table 3. These observations agreed with earlier studies by Chukwu, et al. [19]; Ojokoh, et al. [20] and Ojokoh and Orekoya [15].

Table 3. Occurrence of bacterial and fungal isolates during the fermentation of watermelon seed for in the production of condiment.

Name of organism	Day 1	Day 2	Day3	Day4	Day5	Day6	Day7	Day8	Day 9
Alcaligenes sp	-	-	+	+	+	+	+	+	+
Bacillus subtilis	+	+	+	+	+	+	+	+	+
Bacillus pumilis	-	+	+	+	+	+	+	+	+
Bacillus firmus	-	-	+	+	+	-	+	-	-
Bacillus megaterium	-	+	+	+	+	+	+	+	+
Candida krusei	+	+	+	+	+	+	+	+	+
Candida tropicalis	-	+	+	+	+	+	+	+	+
Fusarium verticuliodes	-	-	+	+	+	-	-	-	-
Lactococcus lactis	+	+	+	+	-	-	-	-	-
Micrococcus sp	+	-	+	+	+	-	-	-	-
Mucor hiamalis	+	+	+	+	+	-	-	-	-
Pseudomonas sp	-	-	+	+	+	+	+	+	+
Pediococcus sp	-	-	+	+	+	-	-	-	-
Rhyzopus stolonifera	+	+	+	+	+	-	-	-	-
Saccharomyces sp	+	+	+	+	+	+	+	+	+
Streptococcus lactis	+	+	+	-	-	-	-	-	-

Note: Key: + Present, - Absent.

Table 4. Physio-chemical parameter of watermelon seeds fermented for the production of a condiment.

DAYS	pH	TTA (%)	TEMP. (°C)
1	6.3	0.176	29
2	6.6	0.149	32
3	7.2	0.104	33
4	7.6	0.099	36
5	7.8	0.081	35
6	8.1	0.068	36
7	8.2	0.059	37
8	8.4	0.045	39
9	8.7	0.027	40

The organoleptic characteristics of the fermented sample was compared to ogiri sample bought from the market Figure 1 Generally, the colour of the laboratory fermented sample was darker, with a stronger ammonia odour [21-23] in all the panelist preferred the fermented sample to the sample bought from the market. Therefore, the fermented sample was adjudged like very much while the sample bought from the market was adjudged like moderately.

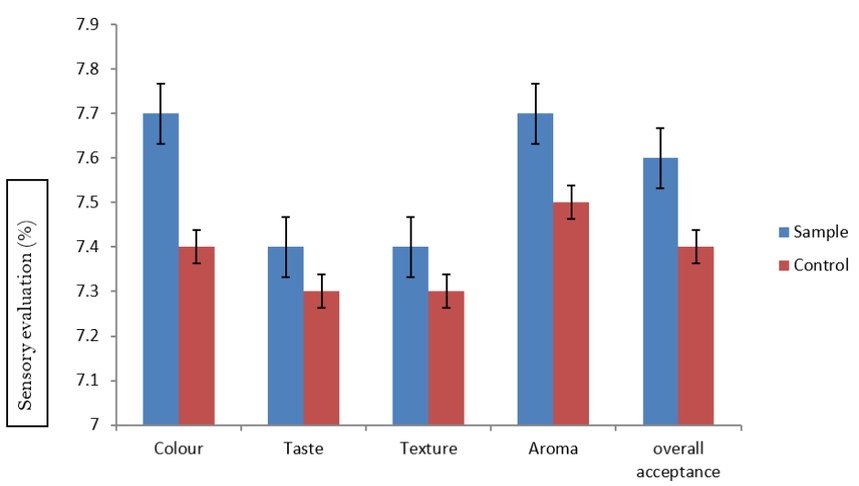

Figure 1. Sensory properties of fermented watermelon seed and commercial condiment.

5. Conclusion

The results from this study revealed that fermented condiment produced from watermelon seed was more accepted by the test panel. This unconventional substrate could therefore serve as a good alternative for the production of ogiri, a local condiment.

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