Effect of Pre-treatment Methods on the Quality Characteristics of Stored *Irvingia* kernel

Fidelis Azi¹*, F. C. Ogbo², Amechi S. Nwankwegu², Michael O. Odo² and Martin O. Anagboso²

¹Department of Food Science and Technology, Faculty of Agriculture and Natural Resources Management, Ebonyi State University, Abakaliki, P.O.Box 053, Abakaliki, Ebonyi State, Nigeria.

²Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, P.M.B 5025, Awka, Anambra State, Nigeria.

Authors’ contributions

This work was carried out in collaboration between all authors. Author FA designed the study and performed the statistical analysis. Author FCO wrote the protocol and the first draft of the manuscript while authors ASN, MOO and MOA managed literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/23314

Editorial:
(1) Giuseppe Blaiotta, Department of Food Science, Via Università, Italy.

Reviewers:
(1) Fernando José Cebola Lidon, New University of Lisobn, Portugal.
(2) Mezaini Abdelkader, Chief University, Algeria.
(3) Małgorzata Gniewosz, Warsaw University of Life Science, Poland.

Complete Peer review History: http://sciencedomain.org/review-history/14583

ABSTRACT

**Aims:** To evaluate effects of pre-treatment methods on the keeping quality of stored *Irvingia* kernels using ethanol and Ash from palm fronds as preservative agents.

**Study Design:** Study of the microbial isolation using sabouraud dextrose agar and nutrient agar, introduction of these microbial isolates on the differently treated freshly harvested *Irvingia* kernels under experimental conditions.

**Place and Study Duration:** Department of Applied Microbiology and Brewing Nnamdi Azikiwe University, PMB 5025, Awka, Anambra state, Nigeria between March, 2013 to September, 2014.

**Methodology:** Sample collection, kernel extraction, treatment with Ash and ethanol and storage in a storage shelf for a period of six (6) months then microbial enumerations and chemical analyses. Effectiveness of each preservative agent were assayed both chemically and microbiologically.

**Results:** Results showed that the pre-treated samples had better quality characteristics than that of
1. INTRODUCTION

Irvingia species (I. gabonensis and I. wombulu) popularly referred to as “Ogbono” among some ethnic regions of Nigeria is a highly economically and nutritionally important tree native to most tropical forest in west and central Africa as well as South-East Asia [1]. This tree is most valued for its fat and protein rich kernels as well as its rich dietary fibre. The kernel is extensively utilized as soup thickener especially in developing countries. In addition to its nutritional benefits, I. gabonensis is highly valued for its health and medicinal benefits. For instance, recent studies have revealed that the dietary fibre present in Irvingia kernels has the ability to reduce the hyperglycemic effects and lipid metabolism disruption caused by diabetes mellitus [2]. Irvingia kernels and its products have also been shown to have wide range of industrial applications. These include the use of the fat extracted from the kernel in production of margarine, soap, cosmetics and pharmaceutical products. These uses and applications have made the market for Irvingia kernels very robust and economically viable. However, Irvingia kernels are prone to contamination and spoilage during storage by fungi that are potentially hazardous to both human and animal health.

The spoilage of Irvingia kernel during storage often results in changes in certain functional, chemical and organoleptic properties of the kernels [3]. These changes subsequently result in significant reduction in both the economic and nutritional value of the Irvingia kernels. Several studies have shown that Irvingia kernels displayed on shelves for sales in Nigeria markets are often contaminated with spoilage fungi [4]. In particular, they observed that fungal contaminated kernels possess aflatoxin. Aflatoxins are produced primarily by the fungi Aspergillus flavus and A. parasiticus. Health risk from consumption of aflatoxin include acute and chronic liver damage, liver cirrhosis, induction of tumours, neurotoxicity, immunosuppression, embryonic damage, abortion and death [5]. Thus the major setback in the sales and consumption of Irvingia kernels is its susceptibility to post harvest spoilage fungi with its attendant health risk. This research was carried to develop more effective pre-treatment methods for preservation of Irvingia gabonensis using substances which are locally available and affordable to farmers. And to also determine the influence of the pre-treatment methods on Irvingia gabonensis spoilage fungi, evaluate the effect of the various pre-treatment methods on the quality characteristics of the stored Irvingia kernel such as drawability, visual colour changes and concentration of Free Fatty Acid as well as ascertain the shelf life of the preserved Irvingia kernels, isolate and identify microorganisms responsible for the spoilage of Irvingia kernel.

2. MATERIALS AND METHODS

2.1 Sample Collection

Ogbono (Irvingia gabonensis) fruits were purchased from local market in Agbaja Izi Local Government Area of Ebonyi State, Nigeria. A local farmer that specialized in extraction and processing of Irvingia kernel was contracted to...
extract the kernel. The extracted kernels were subsequently sorted into grades. The kernels that met the grade “A” requirement were used for this study [6-8].

2.2 Irvingia kernel Pre-treatment

The extracted kernels were divided into three sets, the first two sets were immediately pre-treated with Ash and Ethanol (70% alcohol) while the third set was left untreated as control. Kernels (about half a kilogram) that received different pre-treatment were sealed separately in jute bags before storage.

2.3 Pre-treatment with Ash

Ash from palm-fronds was used; 40 g of powdered ash was used to pre-treat 400 g of Irvingia kernels. The powdered ash was sprayed on freshly harvested Irvingia kernels using a manual hand sprayer and sun dried.

2.4 Pre-treatment Ethanol

Ethanol (40 ml) containing 70% alcohol was used to pre-treat 400 g of freshly harvested Irvingia kernels. The ethanol was sprayed on the kernels, sun dried and stored.

2.5 Storage Conditions

The samples were stored on shelves, indoors. Room temperature was between 27°C and 29°C and relative humidity of 71% - 74%, during the storage period of six months.

2.6 Analysis for Visual Quality Characteristics of the Irvingia kernel during Storage

The Irvingia kernels were monitored for any visual colour changes during the storage period. The changes in colour were examined and observations recorded monthly throughout the storage period.

2.7 Extractable Colour Measurement of the Stored Irvingia kernel using Spectrophotometer

The determination was carried out according to the method proposed by the American spice trade association (ASTA) [9]. The aliquot of the sample solution was used for the spectrophotometric measurement at 460 nm. The absorbance was recorded as displayed on the spectrophotometric screen.

2.8 Determination of Viscosity of the Stored Irvingia kernel

The viscosity of the stored Irvingia kernel was determined as viscosity with the aid of a Rotary digital viscometer (NDJ – 85) China using spindle 2 at 30 rpm. The mucilage from the Irvingia kernels was extracted with the boiling water at 100°C using Irvingia kernel flour to water ratio of 1:40 (W/V). The extraction was carried out by stirring the mixture. The mixture was left to cool at room temperature. The mixture was then centrifuged at 4500 rpm for 30 min and filtered through cotton wool. The extract was transferred into a beaker and placed on the rotating spindle and the values of the viscosity of the extract from the Irvingia kernel displayed on the LCD screen was read in paschal per second (Pa.S).

2.9 Analysis for Chemical Quality Characteristics of the Irvingia kernel during Storage

2.9.1 pH determination

The pH of the samples was determined using highly sensitive digital pH meter (Montini 095, Romania).

2.9.2 Determination of concentration of Free Fatty Acid (FFA) of the stored Irvingia kernel

The concentration of FFA was determined using the standard analytical methods for fats and oils as recommended by American oil chemists’ society (AOCS) [9]. The oil was first extracted from the ground Irvingia kernel using petroleum ether. One gram of the extracted oil was used to determine the concentration of free fatty acid in the sample. One gram of the extracted oil was measured into a 250 ml conical flask and 25 ml of absolute ethanol (99.5% w/v) was also measured and added. Two drops of phenolphthalein indicator was added and the titration was done with 0.1 M NaOH. The percentage FFA value was calculated from the equation below.

\[ A = \frac{V \times M \times W}{m} \times 100 \]

Where

A is the % FFA
V is the volume of NaOH used (ml)
M is the molarity of the NaOH used (mol/1000 ml)
W is the average molecular weight of the fatty acid (myristic acid) component in the oil
\( m \) is the mass of the extracted *Irvingia* kernel oil used.
The analysis was repeated three times and results recorded.

### 2.9.3 Total viable bacterial and fungal count

Ten-fold serial dilution and pour plate method were used for both fungal and bacterial count. All media used (Nutrient agar and Saboraud Dextrose Agar) were prepared according to manufacturer’s instruction (BIOTECH India) and autoclave for 15 minutes at 121°C and 15 psi. The prepared media was allowed to cool to about 40°C in a water bath and was then poured into sterile petri-dishes containing 1 ml aliquot of the appropriate dilutions (normal saline as diluents) prepared from the samples. The samples solutions were prepared by adding 1 g of the sample into 10 ml of normal saline. The plates were incubated for 3 days at room temperature and colonies formed were counted and expressed in colony forming unit per gram CFU/g.

### 2.9.4 Fungal isolation

The different fungal colonies growing on the viable count plates were subcultured on Saboraud Dextrose Agar (SDA) and incubated at room temperature of 25°C for 3 days.

### 2.9.5 Fungal identification

The fungal colonies subcultured on Saboraud Dextrose Agar (SDA) were identified based on colony morphology, microscopic morphology and comparison with different fungal Atlases [10, 11].

### 2.9.6 Molecular identification of fungal isolates

The isolates were further identified to species level at CABI Microbial Identification Services (United Kingdom, Bakem Lane, Egham Surrey TW20 9TY, UK) where internally transcribe spacer (ITS), partial calmodulin and transcriptional elongation factor (TEF) rDNA sequencing analyses were used for the identification of the fungal isolates. A unique CABI reference number (IMI number) was assigned to each of the isolates (IMI 504738, IMI 504739, IMI 504740, IMI 504741, and IMI 504742).

### 2.9.7 Aflatoxin analysis

Determination of total aflatoxin on the *Irvingia* samples was done by the use of Enzyme link immunosorbent assay (ELISA) Method. Extraction of the aflatoxin was done with Tween-ethanol. The sample was first ground into fine powder. Twenty five millilitre of Tween-ethanol was added to 5 g of the sample and mixed properly. The sample solution was then centrifuged at 250 rpm for 3 mins. The centrifuged sample was filtered with Watman1 filter paper.

Aflatoxin conjugate (200 micro liter) was dropped in a clean mixing wall and 100 microliter of the sample analyte was added. The mixture of the aflatoxin conjugate and the sample was then transferred into antibody incubated micro-walls and incubated under dark cover at room temperature for 15 mins. This process was allowed for the antibody/antigen reaction to take place. After the incubation the solution was then washed off 5 times using deionized water and then 100 microliter of the substrate was added and allowed to stand for 5 mins. Finally a stop solution was added and the result read with ELISA machine.

### 2.10 Statistical Analysis

The statistical analyses were carried out using IBM SPSS program. P-values test of significance was carried out at 95% level of confidence. Analysis of variance (ANOVA) was performed using SPSS 16.0 version.

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of Pre-treatment on the *Irvingia* kernel Fungal Population

Result showed that the control sample had the highest fungal population followed by sample pre-treated with ash while the sample pre-treated with ethanol had the lowest fungal population at the end of the storage period (Fig. 1). This implied that the pre-treatment agents had effect on the fungal quality of the *Irvingia* kernel during storage. This result is similar to the findings of Ebimieowei and Dorcas, [4] in which they reported that the fungal populations of stored *Irvingia* kernels were greatly influenced by pre-treatment with 0.9%NaCl and 3%KHCO₃.
The result of the fungal isolation and identification showed that the control sample had *Aspergillus flavus*, *Eurotium cristatum*, *Eurotium chevalier* and *Neurospora sp*. The sample pre-treated with ash had *Eurotium cristatum*, *Eurotium chevalier* and *Neurospora sp*. While the sample pre-treated with ethanol had only *Eurotium cristatum* and *Eurotium chevalier*.

Pre-treatment with ethanol inhibited the growth of *Aspergillus flavus*, *Aspergillus niger* and *Neurospora sp* while pre-treatment with Ash inhibited the growth of *Aspergillus flavus* and *Aspergillus niger*. Thus *Aspergillus flavus* that is known to produce aflatoxin was only isolated on the control sample but was inhibited in both pre-treated options. Thus pre-treatment with ethanol had the highest inhibitory effect on the *Irvingia* kernel spoilage fungi as only *Eurotium* species were isolated from the sample at the end of the storage period.

3.2 Pre-treatment Effect on *Irvingia* kernel Free Fatty Acid Concentration

Result from the determination of the concentration of free fatty acid (FFA) in the *Irvingia* kernel revealed that the control sample had the highest concentration of FFA (5.08%), followed by sample pre-treated with ash (2.82%) while the sample pre-treated with ethanol had the lowest concentration of FFA (2.26%). It then follows that pre-treatment with ethanol was most effective in significantly reducing the rapid lipid hydrolysis and rancidity often associated with stored *Irvingia* kernels. It has been reported that the acidity and a rancid taste often begin to be noticeable in foods when the concentration of free fatty acid is about 0.5 to 1.5% [3]. The maximum acceptable level of FFA in crude extracted oil from plant source meant for domestic consumption has been recommended to be between 0.0 and 3% [9]. This is because consumption of food high in free fatty acids has been widely reported to have direct link with cardiovascular diseases [8]. Findings from this research showed that pre-treatment with ethanol was able to maintain the concentration of free fatty acid below 3.0% throughout the storage period. This suggests that ethanol could be a very effective substance for preservation of *Irvingia* kernel before storage (Table 1).

3.3 Effect of Pre-treatments on the Viscosity of the *Irvingia* kernel

Drawability is one of the most desired quality characteristics of *Irvingia* kernel. In this research drawability was measured as viscosity and result showed that the sample pre-treated with ethanol had a better drawing ability than the sample pre-treated with ash while the control sample had the least drawability at the end of the storage period (Table 2). However there was general decrease in drawability of the *Irvingia* kernel as the storage time increased. This is similar to a work done by Akusu et al. [3] in which they reported a general decrease in viscosity (drawability) of ogbono flour over a storage period of six weeks. However there was variation in the viscosity of the different *Irvingia* samples from the beginning of the storage. This could be as a result of the effect
of the pre-treatments on the Irvingia samples within the drying period of 3 weeks before storage.

3.4 Effect of Pre-treatments on the Colour of the Irvingia kernel

Visual colour changes studied revealed that the untreated sample (control) was the most discoloured while the sample pre-treated with ethanol was the least discoloured at the end of the storage period (Table 3). This may be due to increased microbial activities in the control sample as microbial spoilage of Irvingia kernel has been established to be responsible for rapid discoloration of stored Irvingia kernel (1). The result further showed that between the pre-treated samples, sample pre-treated with ethanol had the best visual colour characteristics at the end of the storage period. This is similar to the work of Ebimieowei and Dorcas, [4] in which they reported a good visual quality of Irvingia kernel pre-treated with 0.9% NaCl solution over a storage period of 3 months.

Table 1. Changes in free fatty acid concentration at 27°C-29°C (RH 71-74%) (%)

| Samples | 0 month | 2 month | 4 month | 6 month |
|---------|---------|---------|---------|---------|
| Control | 2.26±0.030<sup>a</sup> | 3.10±0.100<sup>a</sup> | 3.67±0.070<sup>a</sup> | 5.08±0.080<sup>a</sup> |
| Ethanol | 0.28±0.070<sup>b</sup> | 0.28±0.020<sup>c</sup> | 1.13±0.030<sup>c</sup> | 2.26±0.030<sup>c</sup> |
| Ash     | 0.56±0.010<sup>d</sup> | 0.85±0.040<sup>d</sup> | 1.69±0.020<sup>d</sup> | 2.82±0.020<sup>d</sup> |

<sup>*Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the column are significantly different (p<0.05)</sup>

Table 2. Effect of pre-treatments on the viscosity of the Irvingia kernel (Pa.s)

| Samples | 0 months | 2 month | 4 month | 6 month |
|---------|----------|---------|---------|---------|
| Control | 0.232±0.419<sup>a</sup> | 0.201±0.001<sup>a</sup> | 0.213±0.013<sup>a</sup> | 0.192±0.100<sup>a</sup> |
| Ethanol | 0.401±0.096<sup>c</sup> | 0.400±0.010<sup>c</sup> | 0.382±0.001<sup>c</sup> | 0.299±0.099<sup>c</sup> |
| Ash     | 0.270±0.033<sup>d</sup> | 0.259±0.016<sup>d</sup> | 0.216±0.016<sup>d</sup> | 0.203±0.011<sup>d</sup> |

<sup>*Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the column are significantly different (p<0.05)</sup>

Table 3. Visual colour changes of the Irvingia kernel during storage

| Samples | 0 month | 2 month | 4 month | 6 month |
|---------|---------|---------|---------|---------|
| Samples | Creamy White | Creamy/yellow | Dark brown | Blackish |
| Ethanol | Creamy White | Creamy White | Creamy White | pale yellow |
| Ash     | Creamy White | Creamy White | Pale green | Dark |

3.5 Total Aflatoxin Determination

The quantity of aflatoxin in the Irvingia sample (control) was above the 10 ppb maximum permissible limit in food as recommended by the
National Agency Food and Drug Administration and Control (NAFDAC) (Table 5). This portends a great health risk for consumers of Irvingia kernel as ingestion of this level of aflatoxin in food has been directly linked with liver cancer or even acute death (4). However, the sample pre-treated with ethanol and ash had no aflatoxin content.

Table 5. Total aflatoxin determination

| Samples | Aflatoxin |
|---------|-----------|
| Control | 13.9 ppb  |
| Ethanol | 0.00 ppb  |
| Ash     | 0.00 ppb  |

4. CONCLUSION

Results from this work showed that pre-treatment of Irvingia kernel with ethanol before storage significantly improved the visual and chemical quality characteristics of the Irvingia kernel during the storage. Pre – treatment with the ethanol also reduced the fungal populations of the stored Irvingia kernels and inhibited the growth of Aspergillus flavus which is a known aflatoxin producer. It is therefore recommended that Irvingia kernel be pre – treated with ethanol before storage as this will not only improve the storage quality of the Irvingia kernel but will also guarantee health and safety of the people that consume the kernel.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ikhatua ML, Adewumi ERK, Nassang AL. Microbial spoilage of Irvingia Kernels in Benin City. Archives of Applied Science Research. 2010;2(5):168–78.
2. Lesley TB. Potential for novel food products from agroforestry trees: A review. Food Chemistry. 1996;66:1–14.
3. Akusu OM, Kiin-Kabari D. Effect of storage period on selected functional and chemical stability and sensory properties of bush mango (Irvingiagabonenesis) seed floor. Africa Journal of Food Science and Technology. 2013;4(6):136–40.
4. Ebimieowie E, Dorcas DSB. The effect of treatment methods and storage conditions on postharvest disease and fungal quality of Irvingia gabonensis. J. Food Science and Quality Management. 2012;10:2224–6088.
5. Dorner JW, Cole RJ, Lomax LG. Cyclopiazonic acid production by Aspergillus flavus and its effects on broiler chickens. Appl. Environ. Microbiol. 1999;46:698–703.
6. Ladipo DO. Development of quality control standards for ogbono (Irvingia gabonensis and Irvingia wombula) kernels: Efforts towards encouraging organized and further international trade in West and Central Africa. Food and Agriculture Organization Corporate Repository. 2012;6:56–67.
7. AOCS. Official methods and recommended practices of the American Oil Chemists’ Society 2004. 5th ed. Champaign, Ill: AOCS (Method Cd 8-53 and Ca 5a-40).
8. Babalola TOO, Apata DF. Chemical and quality evaluation of some alternative lipid sources for aqua feed production. Agriculture and Biology Journal of North America. 2011;2(6):935–43.
9. ASTA. Official of methods and recommended practices of the American Spice Trade Association; 2006. Washington DC, USA.
10. DeHoog GS, Guarro J, Gene J, Figueras MJ A. Atlas of clinical mycology; 2004. Atlas Version.
11. Adebayo-Tayo B, Onilude C, Ogunjobi AA, Gbolagade JS, Oladapo MO. Determination of fungi and aflatoxin in shelved bush mango seeds (Irvingia spp.) stored for sale in Uyo, Nigeria. African Journal of Biotechnology. 2006;5(19):1729–1732.

© 2016 Azi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14583