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

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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.

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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 *Invingia 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

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the control sample at the end of the storage period. Fungal population of the pre-treated samples differed significantly with the untreated sample (control) at ($P<0.05$). Total aflatoxin analysis carried out on the samples was positive for the control sample only thus, depicted a contamination by *Aspergillus flavus* which was identified in the control sample. Aflatoxin concentration of 13.90 ppb was reported. This is above the National Agency for Food and Drugs administration and Control, Nigeria (NAFDAC) recommended maximum permissible level in food (10 ppb). Zero bacterial counts upon enumeration using nutrient agar was reported in both treated and untreated samples and therefore, suggested that preponderance of the *Irvingia* kernels post- harvest spoilage organisms are fungi.

Conclusion: Results from this work showed that pre-treatment of *Irvingia* kernel with ethanol before storage significantly improved the chemical and microbial 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.

Keywords: Invirgia kernel; pre-treatment; fungi; afflatoxin; spoilage; shelf-life.

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 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 Izzi 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 rmp 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 15minutes at 121'^{C} and 15psi. 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 comparision 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 504737, 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 donewith Tween-ethanol.The sample was first ground into fine powder. Twenty five mililitre 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 5mins. 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%$KHCO_3$.

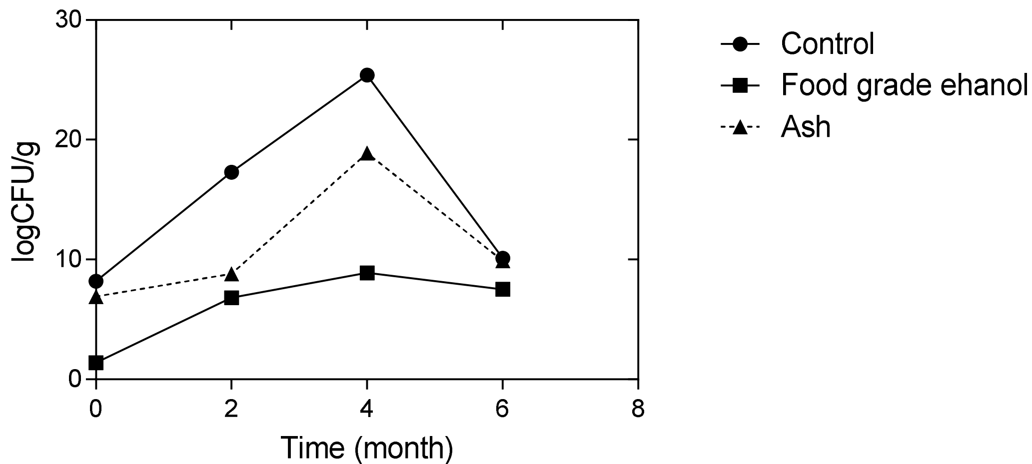

Fig. 1. Fungal population versus time in month

[q]Solid circle- Control; solid square- Food grade ethanol and solid triangle- Ash

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 Neurosporasp* 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* sampls 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 discolouration 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.

The result of Colour changes determination using spectrophotometer collaborated with the result of visual colouration (Table 4). The control sample had the highest absorbance indicating higher colouration while sample pre – treated with ethanol had the least absorbance implying least colouration at the end of the storage period. This finding is similar to the report of Ladipo, [6] in which he reported that many *Irvingia* kernels displayed on shelves for sale in Nigeria market are contaminated with spoilage fungi which often grow and impact different colours on the *Irvingia* kernel during storage.

3.5 Total Aflatoxin Dtetermination

The quantity of aflatoxin in the *Irvingia* sample (control) was above the 10 ppb maximum permissible limit in food as recommended by the

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 ^{a}	3.10±0.100 ^{a}	3.67±0.070 ^{a}	5.08±0.080 ^{a}
Ethanol	0.28±0.070 ^{b}	0.28±0.020 ^{e}	1.13±0.030 ^{e}	2.26±0.030 ^{e}
Ash	0.56±0.010 ^{d}	0.85±0.040 ^{d}	1.69±0.020 ^{d}	2.82±0.020 ^{d}

[a]Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the column are significantly different (p<0.05)

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 ^{a}	0.201±0.001 ^{a}	0.213±0.013 ^{a}	0.192±0.100 ^{a}
Ethanol	0.401±0.096 ^{c}	0.400±0.010 ^{c}	0.382±0.001 ^{c}	0.299±0.099 ^{c}
Ash	0.270±0.033 ^{e}	0.259±0.0.016 ^{e}	0.216±0.016 ^{e}	0.203±0.011 ^{e}

[a]Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the column are significantly different (p<0.05)

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

Table 4. Colour changes (Absorbance at 460 nm)

Samples	0 month	2 month	4 month	6 month
Control	0.120±0.010 ^{b}	0.331±0.001 ^{b}	0.876±0.001 ^{b}	1.801±0.001 ^{b}
Ethanol	0.046±0.001 ^{c}	0.091±0.002 ^{c}	0.124±0.564 ^{c}	1.021±0.021 ^{c}
Ash	0.157±0.007 ^{d}	0.254±0.005 ^{e}	0.502±0.001 ^{e}	1.487±0.005 ^{e}

[x]Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the column are significantly different (p<0.05)

National Agency Food and Drug Administration and Control (NAFDAC) (Table 5). This portend a great health risk for consumers of *Irvingia* kernel as ingestion of this level of aflatoxin in food have been directly link 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.

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