EFFECTS OF WRAPPING MATERIALS ON MOULD GROWTH AND PROXIMATE COMPOSITION OF *PENTACLETHRA MACROPHYLLA* BENTH

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**ABSTRACT**
Studies on the effects of wrapping materials on mould growth and the proximate composition of *Pentaclethra macrophylla* Benth (oil bean) were carried out in the Plant Pathology and Food Science and Technology Laboratories in Rivers State University of Science and Technology, Port Harcourt respectively. The two different wrapping materials used were the leaves of *Theumatococcus danielli* (a local leave) and the synthetic cellophane. It was observed that processed oil bean seed wrapped with the local leaves harbored more moulds than those wrapped with the cellophane. All the fungal isolates were found to be pathogenic to freshly processed oil bean seeds causing general soft rot. The assessed proximate values comprising the ash, protein, carbohydrate, lipids and fiber were higher in samples wrapped with the local leaves with the exception of moisture values which were higher in samples wrapped with cellophane.

**Key words:** Wrapping materials, moulds, proximate composition and *Pentaclethra macrophylla*.

**INTRODUCTION:**
Oil bean popularly known as ugba is a fermented condiment from the seeds of African oil bean tree *Pentaclethra macrophylla* Benth. The oil bean tree which is an ever green tropical forest tree is indigenous to Nigeria. It contains about 50% (w/w) oil and 18% (w/w) protein. The oil from the seed is classified as inedible seed meal because of its content of ricin and other toxic constituents (Amachree, 1997). However, a seed product called ugba which is obtained after detoxification through fermentation can be used in a variety of ways. Ugba is commonly eaten and highly cherished by the Igbos in the Eastern part of Nigeria who serve it in mixture with stock fish during ceremonies (Amachree, 1997). It has also become popular in Rivers State s a major condiment of the special menu called “African salad”. The methods of the
preparation of ugba vary slightly from place
to place. However, they are essentially as
described by (Obeta, 1983). The seeds are
boiled in water for about 16-18 hours and
the tough black fibrous testae are dehulled
by breaking the shell slightly to expose the
cotyledons. The cotyledons are cut into thin
slices of various sizes and thickness, and
later boiled for 30 minutes and soaked for
some time at ambient temperature of (28C -
30C). Boiling reduces the bitter taste. The
slices are then washed thoroughly with tap
water and wrapped with some local leaves
or cellophane for some days to ensure
proper fermentation before consumption.
The short shelf-life of the product is one of
the factors limiting its production on a large
scale (Amachree, 1997). Several macro and
micro organisms are responsible for the
spoilage of processed oil bean seeds such as
Aspergillus spp, Mucor mucedo and
Rhizopus spp.(Njoku and Okwelle, 1992).
This work therefore was carried out to
determine the effects of wrapping materials
on mould incidence and the proximate
composition of processed oil bean seeds
purchased from different markets in Port
Harcourt. The knowledge of this will proffer
a solution to the control of these moulds and
will suggest the best material for wrapping
of the processed seed of oil bean.

Freshly processed oil bean seeds wrapped
with leaves of Theumatococcus danielli and
cellophane were purchased from five
different markets in Port Harcourt. The
freshness of the processed seeds was
ascertained by physically untieing the
wrapped processed seeds and chewing the
condiment both in the local leaves and the
cellophane. The samples were transported
quickly from the various markets within 30
minutes to the Plant Pathology Laboratory
for further studies.

Proximate composition determination of
processed seeds wrapped with different
materials from different locations.
Processed oil bean seeds from five markets
namely (Mile 1, Mile 3, Rumuokwuta
market, Rumuokoro and Choba market)
wrapped with the local leaves and
cellophane were taken to the Food Science
and Technology Laboratory for the
determination of their proximate
compositions comprising of ash, protein,
moisture, lipid, carbohydrate and fibre.
These parameters were determined
according to the method of Association of

Mycological studies
Preparation of test materials, glass wares
and other equipment
Preliminary operations performed in
preparation for isolation included the
sterilization of conical flasks, slides and
Petri dishes previously washed with soap

MATERIALS AND METHODS
Collection of samples
and water. Sterilization of the glass wares was done according to the method of (Agrios, 2005). Inoculating loops were sterilized by dipping for 1 minute in 70-80% ethyl alcohol and heated over a Bunsen flame to red hot.

**Media preparation**
The medium used for fungi isolation was the Sabouraud Dextrose Agar (SDA). This was prepared by weighing 32.8g of Sabouraud Dextrose Agar (SDA) into a 500ml conical flask. Distilled water (500ml) was added into the flask with a measuring cylinder and stirred to homogenize. The mouth of the conical flask was plugged with sterile cotton wool and wrapped with foil. The conical flask with its contents was autoclaved for 15 minutes at 121°C at 1.1kg cm⁻³ pressure. Sterile Petri dishes were prepared and the mixture dispensed into them while still hot and allowed to solidify.

**Isolation and identification of fungi from processed and fermented seeds of P. macrophylla Benth wrapped with local leaves and cellophane.**
Fermented samples of oil bean seeds wrapped with local leaves and cellophane from the various markets were washed in tap water, rinsed in distilled water and surface sterilized in 70% ethyl alcohol. Five slices from each wrap were introduced into the Sabouraud Dextrose Agar (SDA) in Petri dishes with the help of a hand forceps in triplicate. The inoculated plates and their contents were incubated for 7 days at room temperature of 25±3°C. Pure cultures of fungi growing in mixtures were obtained thereafter. Fungal spores from pure cultures were picked with sterile needle and placed on sterile slides. The spores were carefully teased to ensure proper spread on the slides. The properly spread spores were stained with lactophenol in cotton blue and examined microscopically using various magnifications. This procedure was repeated for all the fungal isolates from different markets. The fungi were identified based on their colonial morphology, mycelia structure, spores and associated structures according to the keys of (Olds, 1983).

**Pathogenicity studies**
Pathogenicity test was carried out to determine if the fungal isolates responsible for the spoilage of processed and fermented oil bean seeds were capable of causing rot of freshly processed samples. The procedure described by (Agrios, 2005) and (Trigiano et al, 2004) was used. Boiled and sliced seeds of oil bean were prepared and surface sterilized with 70% ethyl alcohol. Each of the fungal isolates was aseptically transferred onto the tin slices of oil bean seeds on damp blotter papers in Petri dishes and incubated at room temperature of 25±3°C for 5 days. Petri dishes containing sliced oil bean samples without the fungal isolates served as control for each of the markets.
Data generated from the proximate composition analysis were interpreted using percentages and standard error, while the results of the fungal isolates were interpreted using percentages.

RESULTS AND DISCUSSION

The results of the proximate composition of the processed seeds of *P. macrophylla* Benth wrapped with the leaves of *T. danielli* and cellophane are presented in Table 1. Processed oil bean seeds from Mile 3 market wrapped with local leaves and cellophane recorded the following values for ash (2.5 and 2.4), moisture (12.4 and 13.2), fat (31.5 and 31.1), fibre (2.6 and 2.7), carbohydrate (10.3 and 15.5) and protein (40.7 and 35.2) respectively. The protein content of processed oil bean from the Mile 3 market was higher in samples wrapped with local leaves than those wrapped in cellophane. In general, samples wrapped with local leaves recorded higher values for ash, fat and protein while carbohydrate and moisture values were higher in samples wrapped with cellophane. Processed oil bean samples from Choba market recorded higher ash, moisture and protein values from samples wrapped with local leaves while fat, fibre and carbohydrate values were higher in samples wrapped with cellophane. Processed oil bean samples purchased from Rumuokwuta market wrapped with local leaves recorded higher values for ash (2.6), fat (3.9), carbohydrate (15.2) and protein (40.2). Samples wrapped with cellophane had higher values for moisture (13.4) only. Processed oil bean samples from Rumuokoro market recorded higher values for ash (2.6) and protein (36.8) in samples wrapped with local leaves while samples wrapped with cellophane recorded higher values for moisture (13.8), fat (32.5), fibre (3.0) and carbohydrate (14.3). Samples from Mile 1 market recorded the following values for samples wrapped with local leaves and cellophane ash (2.6 and 2.4), moisture (13.1 and 14.8), fat (31.4 and 33.6), fibre (2.65 and 3.10) carbohydrate (12.9 and 11.9) and protein (37.8 and 35.2). In general, moisture content was higher in samples wrapped with cellophane than in samples wrapped with local leaves. Moisture values from processed oil bean wrapped in cellophane was higher from four out of the five markets sampled. Ash and protein values were higher in samples wrapped with local leaves in all the markets indicating that local leaves helped in concentrating ash and proteins which are essential food elements. Fibre values were higher in samples wrapped in cellophane in all the markets. Variable values were obtained for fats from samples purchased from the different markets. Higher carbohydrate values were recorded mostly from samples wrapped with cellophane in most of the markets. The methods of processing may have affected some of the test samples either positively or negatively in addition to the wrapping materials.
Table 1: Proximate composition of processed oil bean wrapped with local leaves and cellophane from different market.

<table>
<thead>
<tr>
<th>Location</th>
<th>Wrapping material</th>
<th>Ash %</th>
<th>Moisture</th>
<th>Fat %</th>
<th>Fibre%</th>
<th>CHO %</th>
<th>Protein %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mile 3</td>
<td>Leave</td>
<td>2.5± 0.001</td>
<td>12.4± 0.011</td>
<td>31.5±0.033</td>
<td>2.6±0.04</td>
<td>10.3± 0.01</td>
<td>40.7±0.02</td>
</tr>
<tr>
<td></td>
<td>Cellophane</td>
<td>2.4± 0.03</td>
<td>3.2± 0.031</td>
<td>2.7±0.02</td>
<td>15.4±0.02</td>
<td>35.2±0.05</td>
<td></td>
</tr>
<tr>
<td>Choba</td>
<td>Leave</td>
<td>2.5 ± 0.02</td>
<td>12.5±0.031</td>
<td>2.7±0.02</td>
<td>15.4±0.02</td>
<td>40.2 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cellophane</td>
<td>2.3 ± 0.04</td>
<td>2.5 ± 0.02</td>
<td>2.0±0.01</td>
<td>10.1±0.04</td>
<td>34.6 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Rumuokwuta</td>
<td>Leave</td>
<td>2.6 ± 0.03</td>
<td>1.7 ± 0.041</td>
<td>2.6 ± 0.02</td>
<td>2.8 ± 0.01</td>
<td>16.3 ± 0.01</td>
<td>34.6 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Cellophane</td>
<td>2.3 ± 0.01</td>
<td>2.0 ± 0.03</td>
<td>2.0±0.03</td>
<td>2.8 ± 0.01</td>
<td>16.3 ± 0.01</td>
<td>34.6 ± 0.03</td>
</tr>
<tr>
<td>Rumuokoro</td>
<td>Leave</td>
<td>2.6 ± 0.04</td>
<td>13.0 ± 0.021</td>
<td>2.6 ± 0.03</td>
<td>13.2±0.01</td>
<td>36.8 ± 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cellophane</td>
<td>2.3 ± 0.02</td>
<td>3.8±0.01</td>
<td>2.3±0.01</td>
<td>2.5±0.03</td>
<td>14.3±0.02</td>
<td>34.9 ± 0.05</td>
</tr>
<tr>
<td>Mile 1</td>
<td>Leave</td>
<td>2.6 ± 0.03</td>
<td>13.1 ± 0.031</td>
<td>2.7 ± 0.02</td>
<td>12.9±0.02</td>
<td>37.8 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cellophane</td>
<td>2.4 ± 0.02</td>
<td>4.8 ± 0.02</td>
<td>3.1±0.03</td>
<td>11.9±0.02</td>
<td>35.2 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

Standard error = ±

**Fungal isolates of processed oil bean seeds wrapped with local leaves and cellophane from five markets.**

The results of the fungal isolates from processed oil bean seeds wrapped with local leaves and cellophane purchased from five different markets in Port Harcourt are presented in Table 2. Processed oil bean seeds from Choba wrapped with local leaves harbored three fungi with varying degrees of incidence such as *Fusarium oxysporium* (30%), *Aspergillus tamari* (50%) and *Penicillium italicum* (10%), while samples wrapped with cellophane recorded only one fungus, *Sclerotium rolfsii* (20%). Processed samples from Rumuokoro market wrapped with local leaves harbored *F. oxysporium* (30%), *Neurospora crassa* (20%) and *Geotrichum candidum* (40%). Samples wrapped with cellophane recorded only *F. oxysporium* (10%). Processed oil bean seeds from Rumuokwuta market wrapped with local leaves recorded four fungi with varying degrees of incidence such as *N. crassa* (30%), *Rhizopus stolonifer* (30%),
Aspergillus tamari (20%) and Penicillium italicum (50%). Samples wrapped with cellophane recorded Rhizopus stolonifer (20%) and Aspergillus tamari (10%). Oil bean samples bought from Mile 3 market wrapped with local leaves revealed Aspergillus tamari (40%), Penicillium italicum (10%) and Fusarium oxysporium (10%), while samples wrapped with cellophane harbored A. tamari (10%) and F. oxysporium (20%). Furthermore, oil bean samples from Mile 1 market implicated A. tamari (60%), F. oxysporium (60%), P. italicum (30%) and Rhizopus stolonifer (20%), while samples wrapped with cellophane harbored only Geotrichum candidum (50%). Fungi are amongst the most common microorganisms responsible for the deterioration of most agricultural products. These organisms bring about both quality and quantity losses since some of these fungi are field to store pests (Onuegbu, 2002). The isolation of many fungi from processed oil bean seeds wrapped with local leaves is of great concern since most farmers show preference to these leaves than the cellophane. It has been reported that the method of processing, handling and preservation affects the level of contamination and influence the microbial load of agricultural products (Okaka, 1997, Chuku et al, 2009, Achinewhu, 1996). The production processes of oil bean seeds consists of various steps through which they are exposed to microbial contamination from the atmosphere, soil and even dust. These could be the source of fungal contamination during storage. The storage materials used for most of the agricultural produce are known to influence the microbial load of these produce (Ihekoronye and Ngoddy, 1985). The local leaves were also exposed to fungal contamination which may be due to improper washing of the leaves and lack of sterilization before being used for wrapping which must have led to the high fungal load on the samples from local leaves. This result supports the observations of Okaka (1997), Webster (1970), Okhuya and Ayankola (1986). Who reported that spores of Aspergillus spp, Mucor spp and Rhizopus spp are widely distributed in nature. On the proximate analysis, it has been revealed that some nutrient elements are increased due to the type of materials used for their preservation. This variation could also be due to the sources of the oil bean seeds which may have come from different environment.

All the fungal isolates from the processed seeds of oil bean irrespective of the wrapping material used and the markets were found to be pathogenic causing general soft rot of the seeds leading to the decay of the seeds and loss of quality. The fungi re-isolated from the processed oil bean seeds were the same as the initial isolates that were introduced confirming the fungi to be associated with the rot of processed seeds of P. macrophylla. This research has shown that wrapping materials affect mould growth. Further work to access the effect of these moulds on the biochemical properties of P. macrophylla Benth is advocated.
Table 2: Fungal isolates from processed oil bean seeds wrapped with local leave and cellophane from different markets and their incidence.

<table>
<thead>
<tr>
<th>S/No</th>
<th>Locations</th>
<th>Wrapping material</th>
<th>Fungal isolates</th>
<th>% incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Choba</td>
<td>Local Leaves</td>
<td><em>F. oxysporium</em></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. tamari</em></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. italicum</em></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. rolfsii</em></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cellophane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Rumuokoro</td>
<td>Local leaves</td>
<td><em>F. oxysporium</em></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>N. crassa</em></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>G. candidum</em></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cellophane</td>
<td><em>F. oxysporium</em></td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Rumuokwuta</td>
<td>Local Leaves</td>
<td><em>N. crassa</em></td>
<td>30</td>
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<td></td>
<td></td>
<td></td>
<td><em>R. stolonifer</em></td>
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<td><em>A. tamari</em></td>
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<td><em>P. italicum</em></td>
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<td></td>
<td>Cellophane</td>
<td><em>R. stolonifer</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. tamari</em></td>
<td>10</td>
</tr>
<tr>
<td>4.</td>
<td>Mile 3</td>
<td>Local leaves</td>
<td><em>A. tamari</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. italicum</em></td>
<td>10</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. oxysporium</em></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cellophane</td>
<td><em>A. tamari</em></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. oxysporium</em></td>
<td>20</td>
</tr>
<tr>
<td>5.</td>
<td>Mile 1</td>
<td>Local leaves</td>
<td><em>A. tamari</em></td>
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</tr>
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<td></td>
<td><em>F. oxysporium</em></td>
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</tr>
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<td></td>
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<td><em>P. italicum</em></td>
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<td></td>
<td></td>
<td>Cellophane</td>
<td><em>G. candidum</em></td>
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REFERENCES


