Yeasts and Moulds Associated with *ogi*-A Cereal Based Weaning Food During Storage

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**Abstract:** The populations and profiles of moulds and yeasts species present in *ogi* during fermentation and storage at room temperature until spoilage sets in were determined. Yeasts counts increased throughout the fermentation period while moulds were present till 12 h of soaking; thereafter no mould population was observed again. During the storage period, initial yeasts counts (4.62±1.05 log cfu g⁻¹) in the corn steep liquor increased and peaked at 8.96±2.00 log cfu g⁻¹ on day 12, then reduced thereafter. Moulds were not isolated until day 10 and day 12 in the corn steep liquor and the *ogi* samples, respectively. The moulds isolated during storage include *A. niger*, *A. flavus*, *Rhizopus nigricans* and *Penicillium sp.* while the yeasts are *Saccharomyces cerevisiae* (strain 1), *Candida krusei* (strain 2), *C. tropicalis*, *C. vini* (strain 1) and *Geotrichum candidum*. The percentage of occurrence of *A. niger* was 12% on the 8th day, this however increased to 56% by the 20th day. *Saccharomyces cerevisiae* (18%) present at the beginning of storage reduced to 2% by the 10th day of storage while *Candida krusei* (15%) increased to 28% by day 20.

**Key words:** Yeasts, moulds, storage, spoilage, fermentation, *ogi*

**INTRODUCTION**

Lactic acid fermentation of cereal-based foods is a traditional technology in Africa (Mensah, 1997; Oyewole, 1997). Lactic acid, acetic acid and other acids formed during the fermentation process lowers the pH thus inhibiting the growth of most spoilage organisms (Odunfa, 1985; Lorri and Svanberg, 1994; Kingamkurua et al., 1995). *Ogi*-is a cheap and popular weaning food in several West African countries, it also serves as food for convalescence adults. It is produced by lactic acid fermentation of maize, sorghum or millet. Due to the long and tedious processing method, *ogi* is usually prepared in bulk and stored for use. It has been reported that *ogi* can be kept for more than 10 days at room temperature by decanting the sour water and replaced with fresh water 48 hourly or refrigerated. But there are reports that decanting the steeped water results in loss of nutrients (Aremu, 1993). However, when the sour water is not changed, the shelf-life of wet *ogi* is less than 7 days at room temperature (Olasupo et al., 1997).

Despite the delicate health position of some *ogi* consumers and the current knowledge of some toxic metabolites (mycotoxins) produced by fungi, there is little information in literature on all the fungi associated with stored *ogi*. Some available investigations regarding the fungi present in stored *ogi* have concentrated on the moulds. Although yeasts are present during the fermentation of *ogi*, they have generally not been considered as playing major role in spoilage (Odunfa and Adaye, 1985;
Ogunfio et al., 2001). There are dearths of studies reporting populations of yeasts and specific yeast species on stored *ogi* and this makes an accurate assessment of their contribution to spoilage difficult.

*Ogi* is prone to fungal contamination during storage. It is important to identify fungal contaminants in this product because some moulds can grow and produce mycotoxins on these commodities while certain yeasts and moulds can cause infections or allergies. The survey reported here was undertaken to identify the populations and profiles of yeasts and moulds associated with *ogi* during fermentation and storage until spoilage sets in.

**MATERIALS AND METHODS**

**Fermentation and Storage of Ogi**

Dried white maize grains were purchased from the local markets. The grains were processed into *ogi* by steeping in water for 48 h, after which the steep water was drained and the grains wet milled using a double grinding mill (Asiko Engineering, Nigeria). The wet-milled grains were wet sieved as done locally using a hand sieve (Onyejewere et al., 1989). The *ogi* gruel produced was left to settle into *ogi* and corn steep liquor (water) and further fermented for 48 h (souring). The freshly prepared *ogi* was then stored at room temperature without changing the corn steep liquor until spoilage sets in. A total of 20 days storage period was observed. The parameters used to monitor spoilage were as described by Teniola and Ogunfio (2002). The spoilage indices consisted of pH, total titrable acidity, total reducing sugars, dissolved hydrogen sulphide, ammonia level and sensory analysis.

**Microbiological Analysis**

Samples were aseptically taken at 12 h intervals during the first 96 h (48 h soaking and 48 h souring) of fermentation. Similarly, samples of *ogi* and corn steep liquor were taken separately at different intervals during the storage period.

The samples (Corn steep liquor, 10 mL; *ogi*, 10 g) were separately mixed with 90 mL sterile peptone water. Ten fold dilutions were made and aliquots of the appropriate dilutions were plated using the pour plate method. Counts of yeasts and moulds were made respectively on yeast dextrose peptone agar (YEDPA) and Sabouraud dextrose agar (SDA, Oxoid CM 41) containing 50 mg L⁻¹ chloramphenicol and 50 mg L⁻¹ chlorotetacycline to inhibit bacterial growth. Incubation was at 25°C for 2 to 4 days. Colonies were counted and expressed as colony forming units (cfu) per gram for *ogi* samples and per ml for the corn steep liquor. Isolates were purified on Potato dextrose agar (PDA, DIFCO, Detroit, Michigan, USA) and further subcultured for microscopic examination and identification.

**Identification of Isolates**

The purified yeast colonies were subjected to standard tests and classification schemes as described by Barnett et al. (1990). The tests include those for colony and cell morphology, sporulation, fermentation tests and pseudomycelium formation.

Identification of the yeast isolates to species level was done using the ID 32C biochemical kit (Biomerieux, France). Mould identification was performed according to the methods described in Fungi and Food Spoilage (Pitt and Hocking, 1999).

**Sensory Evaluation Test**

A 15 man trained panel who are familiar with *ogi* were asked to assess the qualities of the freshly fermented samples and during storage considering the colour, taste, aroma, appearance and texture. A
five point hedonic scale was used for the evaluation with 5 indicating excellent acceptability for the attributes and 1 indicating a highly characteristic difference from normal or low acceptability. The final score represent the means of all the panelists.

Data Analysis

Analysis of variance (ANOVA) followed by Duncan’s multiple-range test (p<0.05) for the population of moulds and yeasts detected in stored samples was performed by using SPSS 10. Correlation coefficients between the fermentation time and the fungal population were also determined using Pearson bi-variate correlation.

RESULTS

Studies on the changes observed in the parameters used as indices of spoilage during the storage of ogi are presented in Table 1. The pH falls from 4.32±2.00 on the first day of storage to 3.16±2.14 on the 10th day. Thereafter the pH increased till the end of storage. The total reducing sugar declined throughout the storage period. Increases in the levels of dissolved hydrogen sulphide (H₂S) and ammonia were noted until the 16th day of storage after which there were declines. More hydrogen sulphide (H₂S) was produced during storage than ammonia (NH₃). There was a sharp decline in acceptability of the stored ogi between day 6 and day 14 of storage. By the 16th day of storage without changing of water, the ogi sample was totally unacceptable. Discoloration of the water was observed by the 8th day of storage and the discoloration increased with time (data not shown). Analysis of variance (ANOVA) tests indicated that changes in all the parameters studied over time was significant (p<0.05). With the exception of pH, significant correlation (p<0.05) occurred between the total acceptability and the other parameters studied. Correlation between total acceptability and storage time was high, negative and significant at 0.01 level (2- tailed).

Yeast and moulds counts at different stages during the 96 h fermentation is presented in Table 2. Moulds were the predominant population on the maize grains. The yeasts and moulds population on the maize grains were 1.77±0.38 and 6.86±1.68 log cfu g⁻¹, respectively.

<table>
<thead>
<tr>
<th>Storage days</th>
<th>pH±SE*</th>
<th>TTA (%)±SE*</th>
<th>H₂S±SE*</th>
<th>Ammonia±SE*</th>
<th>Reducing sugars±SE*</th>
<th>Total acceptability±SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.32±0.4</td>
<td>0.02±0.0</td>
<td>0±0</td>
<td>0±0</td>
<td>3.2±0.0</td>
<td>9±0.3</td>
</tr>
<tr>
<td>2</td>
<td>3.65±0.3</td>
<td>0.35±0.01</td>
<td>10±0.7</td>
<td>20±1.9</td>
<td>2.8±0.4</td>
<td>8±0.5</td>
</tr>
<tr>
<td>4</td>
<td>3.10±0.4</td>
<td>0.52±0.02</td>
<td>40±1.2</td>
<td>40±3.4</td>
<td>2.6±0.5</td>
<td>7±0.6</td>
</tr>
<tr>
<td>6</td>
<td>3.14±0.2</td>
<td>0.44±0.01</td>
<td>60±2.4</td>
<td>60±3.8</td>
<td>1.5±0.6</td>
<td>7±0.4</td>
</tr>
<tr>
<td>10</td>
<td>3.18±0.2</td>
<td>0.32±0.01</td>
<td>100±6.7</td>
<td>85±2.9</td>
<td>1.2±0.6</td>
<td>5±0.4</td>
</tr>
<tr>
<td>14</td>
<td>3.20±0.3</td>
<td>0.25±0.02</td>
<td>220±5.4</td>
<td>100±5.6</td>
<td>1.0±0.4</td>
<td>2±0.1</td>
</tr>
<tr>
<td>16</td>
<td>3.30±0.5</td>
<td>0.23±0.01</td>
<td>250±4.6</td>
<td>150±4.5</td>
<td>0.8±0.1</td>
<td>0±0.0</td>
</tr>
<tr>
<td>18</td>
<td>3.66±0.6</td>
<td>0.21±0.02</td>
<td>240±4.8</td>
<td>120±4.9</td>
<td>0.6±0.05</td>
<td>0±0.0</td>
</tr>
<tr>
<td>20</td>
<td>3.71±0.4</td>
<td>0.19±0.02</td>
<td>230±3.9</td>
<td>100±3.4</td>
<td>0.4±0.02</td>
<td>0±0.0</td>
</tr>
</tbody>
</table>

*Mean of triplicate determinations±standard error.

Table 2: Changes in yeasts and moulds counts during the fermentation of maize grains for ogi production

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Steeping period</th>
<th>Soaring period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yeast population*</td>
<td>Mould population*</td>
</tr>
<tr>
<td>M**</td>
<td>1.77±0.38</td>
<td>6.86±1.68</td>
</tr>
<tr>
<td>0</td>
<td>2.90±1.90</td>
<td>6.81±1.59</td>
</tr>
<tr>
<td>12</td>
<td>4.73±1.09</td>
<td>3.72±0.09</td>
</tr>
<tr>
<td>24</td>
<td>5.60±1.49</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>36</td>
<td>6.22±1.33</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>48</td>
<td>6.59±2.17</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

*Mean of triplicate determinations (log cfu g⁻¹)±standard error **Maize grains
Yeast isolates were observed throughout the fermentation period while moulds were isolated only during the steeping period. Mean mould population fell significantly (p<0.05) from 6.81±5.60 log CFU g⁻¹ at 0 h to 3.72±4.60 log CFU g⁻¹ at 12 h of soaking and thereafter no mould population was observed again throughout the fermentation period. A general reduction in yeasts population was observed at the beginning of souring and this was followed by continuous increase in yeast population till the end of the souring period. Changes in yeast and mould populations with time were significant (p<0.05). Correlation between mean yeast populations and fermentation time was positive and significant (p<0.01).

The yeasts isolated during the 96 h fermentation period were presumably identified as \textit{Candida vini}, \textit{Candida kruzei}, \textit{Candida tropicalis}, \textit{Saccharomyces cerevisiae}, \textit{Geotrichum candidum}, \textit{G. fermentans} and \textit{Rhodotorula graminis}. The moulds isolated were \textit{Aspergillus niger}, \textit{A. flavus}, \textit{Fusarium subglutinans}, \textit{Rhizopus nigricans} and \textit{Penicillium citrinum}.

Changes in yeasts and moulds populations during storage are presented in Table 3. Yeasts population were present on the samples through out the 20 days storage period. Yeasts population in the corn steep liquor increased with time from 4.62±1.05 on the first day of storage to 8.96±2.00 on the 12th day. Therefore reduction in yeasts population was observed in the water and by the end of the spoilage studies, yeasts population in the water had reduced to 4.72±3.00. Similarly, yeasts population in the \textit{ogi} samples increased from 3.51±0.25 (day 1) to 7.33±1.00 (day 14), therefore, decline in yeasts population was recorded till the end of storage. In contrast, mould were not observed both in the corn steep liquor and the \textit{ogi} samples until the 8 and 10th day, respectively. Mould populations increased steadily with time. Higher moulds and yeasts populations were observed in the steeped water as compared to the \textit{ogi} sample. Changes in yeasts and moulds populations in the water and the \textit{ogi} samples over time were highly significant (p<0.05). Correlations between storage time and fungal populations were significant at 99% confidence interval.

The spectrum, succession and percentage distribution of yeasts and moulds isolated during the 20 days of storage of \textit{ogi} at room temperature are presented in Fig. 1 and 2, respectively.

Although \textit{Rhodotorula} sp. was isolated during the fermentation period, it was not encountered at all through out the storage period. \textit{Candida vini} (strain 1) and \textit{Pichia japonica} were not present during fermentation, they were however isolated during the storage period.

At the beginning of storage, \textit{S. cerevisiae} (strain 1), \textit{C. kruzei} (strain 1), \textit{C. kruzei} (strain 2), \textit{C. tropicalis}, \textit{C. vini} (strain 1) and \textit{G. candidum} were obtained. However, by the 10th day of storage, \textit{S. cerevisiae} (strain 1) was not isolated again. \textit{Candida vini} (strain 2) was observed as from the 4th day of storage and remained throughout the storage period. Similarly, \textit{P. japonica} was isolated by day 10 and remained through out the storage period.

Table 3: Changes in yeasts and mould populations during the storage of \textit{ogi}

<table>
<thead>
<tr>
<th>Storage days</th>
<th>Corn steep liquor</th>
<th>\textit{ogi}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yeast population*</td>
<td>Mould population*</td>
</tr>
<tr>
<td>1</td>
<td>4.62±1.05</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>2</td>
<td>6.92±1.55</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>4</td>
<td>7.93±5.50</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>6</td>
<td>7.96±1.77</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>8</td>
<td>8.84±2.29</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>10</td>
<td>8.85±2.36</td>
<td>2.51±2.00</td>
</tr>
<tr>
<td>12</td>
<td>8.96±2.00</td>
<td>3.02±0.20</td>
</tr>
<tr>
<td>14</td>
<td>8.87±2.00</td>
<td>4.46±1.52</td>
</tr>
<tr>
<td>16</td>
<td>7.87±2.00</td>
<td>5.14±0.60</td>
</tr>
<tr>
<td>18</td>
<td>6.62±2.20</td>
<td>6.82±2.20</td>
</tr>
<tr>
<td>20</td>
<td>4.72±3.00</td>
<td>8.80±2.00</td>
</tr>
</tbody>
</table>

*Mean of triplicate determinations (log CFU g⁻¹)=standard error
Fig. 1: Succession and frequency distribution of yeasts isolates during the storage of 
ogi, SC- S. cerevisiae, CK1-C. krusei (strain 1), C. krusei (strain 2); CV1-C. vini (strain 1); CV2-C. vini (strain 2); GC-G. candidum, GF-G. fermentans; CT-C. tropicalis; PJ-P. japonicum

Fig. 2: Succession and frequency distribution of molds isolates during the storage of 
ogi

At the beginning of storage, about 18% of the yeasts present were different strains of 
S. cerevisiae, however this number was reduced to 2% by the 10th day of storage. The later end of the 
spoilage period was marked by rapid proliferation of different species of Candida and Geotrichum.
Eighteen per-cents (18%) of Candida krusei (strain 2) were present at the beginning of storage and
these increased to 28% by the 20th day of storage. *G. candidum* increased from 13% at the beginning of storage to 26% at the end of the storage period. *C. vini* (strain 2) also increase from 6% on the 6th day of storage to 19% on the 20th day (Fig. 1).

Moulds were not isolated until the 8th day of storage. On the 8th day of storage only *Aspergillus niger* was isolated, however, by the 20th day of storage, the moulds isolated were *Aspergillus niger, Aspergillus flavus, Rhizopus nigricans* and *Penicillium sp*. On the 8th day, the percentage of occurrence of *A. niger* was only 12%, this however increased to 56% by the end of storage.

**DISCUSSION**

The nutritional and organoleptic qualities of fermented products such as *ogi* are usually as a result of the interactions between different organisms. The interactions may be beneficial adding to the final product by means of desirable biochemical changes like the production of aromatic compounds and enzymatic activities (Viljoen, 2001). On the other hand, the interactions may be detrimental causing spoilage by inhibiting the growth of starter cultures and producing off-flavors, or discoloration. In this study, the yeasts and moulds population present during fermentation and storage up to the spoilage stage of *ogi* were isolated and identified.

The presence of moulds such as *Aspergillus niger, Penicillium sp., Rhizopus sp.* on the surfaces of raw maize grains and during the early stage of fermentation has been reported for *ogi* and *kenkey* (Odunfa and Adeyele, 1985; Jespersen *et al.*, 1994). They are the most likely part of the grains surface microflora that is undesirable in many foods because of their mycotoxin producing potentials (Jonsyn, 1989; Jespersen *et al.*, 1994).

The subsequent early elimination of the mould during fermentation is in agreement with other investigations carried out on fermented maize grain for *ogi* and *kenkey* production (Akinrele, 1970; Jespersen *et al.*, 1994; Teniola and Odunfa, 2001). Since lactic acid bacteria are present in high number in the fermentation of maize for *ogi* production, they may contribute to the elimination of the mould population. Bacteria have been shown to suppress the growth of moulds (El-Gendy and Marth, 1980).

The reduction of the yeasts population at the beginning of souring period may be attributed to processing steps such as the replacement of the steeped liquor prior to wet milling, chaff removal as well as the addition of fresh water during the sieving process (Teniola and Odunfa, 2002).

Yeasts are best known for their positive contributions in the fermentation of several products, they can also cause spoilage in a wide range of foods (Fung and Liang, 1990; Rohn et al., 1992). Yeasts are common contaminants of fruit and some dairy products like cream, butter and cheese (Deak and Beauch, 1993; Viljoen, 2001; Restuccia et al., 2006). Results of this study have shown that yeasts are important not only in the fermentation of *ogi* but also in the spoilage as well. Yeasts isolates such as *Candida krusei, C. vini, Geotrichum candidum* and *Pichia japonica* were the dominant yeasts isolated during the storage of *ogi*.

The dominance and proliferation of *Candida sp.* followed by increase in pH and reduction in total acidity of the stored *ogi*. *Candida sp.* has been implicated in decreasing acidity of fermented foods (Nuruda *et al.*, 1995). This contributes to *ogi* spoilage by reducing product acidity and promoting the growth of other spoilage organisms. *Candida vini* has also been reported as spoilage yeasts for some tropical fruit juices and nectars (Tchango et al., 1997).

Moulds were dominant towards the end of the spoilage studies. The presence of mould during the spoilage of *ogi* agrees with the findings of Onyekwere et al. (1989) who reported that the most important organisms that cause spoilage in stored wet *ogi* are *Rhizopus nigricans, Aspergillus* and *Penicillium sp*. These are similar to the spoilage organisms found in market *gari* samples.
(Adeniji and Potter, 1978; Onyekwere et al., 1989). The above three genera are considered the most significant in grains and foods, not only because of their ability to produce many different mycotoxins, such as aflatoxin B1 (AFB1), Fumonisin B1 (FB1), ochratoxin A (OTA), trichotheccenes and zearalenone (ZEN), but also because of their ubiquitous nature. They are toxic to vertebrates including humans and livestock in small concentrations when introduced via a natural route and consequently AFB1, FB1 and OTA were classified as possible carcinogens to humans (Vainio et al., 1993). Following epidemiological studies, AFB1, FB1 and OTA are suspected to be possible determinants of the human diseases hepatocellular carcinoma, oesophageal cancer (Rheeder et al., 1992) and Balkan Endemic Nephropathy (Pohil-Leszkowicz et al., 2002), respectively.

The observed discoloration of the stored ogi coincides with the isolation of moulds on the samples. These findings corroborate the report of Onyekwere et al. (1989) that fungi cause yellowish discoloration and black spots in wet ogi cakes giving the product a fruity offensive odor.

The results of this study showed that moulds and yeasts may play a more prominent role than previously recognized in the fermentation and spoilage of ogi.

REFERENCES


