Microorganisms associated with supernatant solution of fermented maize mash (*omidun*) from two varieties of maize grains

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Abstract: The aim of this work is to isolate and identify microorganisms associated with *omidun,* the supernatant solution of fermented maize mash. Thirty *omidun* samples comprising of fifteen samples each from *ogi* prepared from white maize grains and yellow maize grains were obtained from traditional processors in Abeokuta, Nigeria. Isolation of bacteria and fungi from *omidun* was carried out using De Man Rogosa and Sharpè (MRS) agar and Sabouraud Dextrose Agar (SDA). Pure cultures of the isolates were identified using standard microbiological procedures. The microorganisms most commonly isolated were *Lactobacillus plantarum* (20%), *C. pseudotropicalis* (18.75%), *Candida albicans* (16.25%), *Saccharomyces cerevisiae* (15%), *C. parasilopsis* (13.75%), *C. tropicalis* (12.5%) and *Aspergillus fumigatus* (3.75%). The maize variety has no significant effect ($p>0.05$) on the types of microorganisms present in *omidun*.
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1. Introduction

Fermented foods are of great significance because they provide and preserve vast quantities of nutrients with a wide diversity of flavours, aromas and textures which enrich the human diet. Some of the fermented foods include alcoholic foods/beverages, vinegar, pickled vegetables, cheeses, yoghurts and pastes with meat-like flavours, leavened and sour-dough breads (Steinkraus, 1997). In Nigeria, one common example of indigenous fermented food is *ogi*, smooth texture porridge with sour taste reminiscent of that of yoghurt (Banigo and Muller, 1972). *Ogi* is a popular weaning food for infants, breakfast meal for children and adults as well as a meal of choice for the convalescences and the aged (Teniola and Odunfa, 2001, Omemu *et al.,* 2011).

The traditional preparation of *ogi* include soaking of the maize grains in cold water for 2-3 days, washing severally with clean water, wet milling and sieving to remove bran, hulls and germ (Akinrele, 1970; Odunfa, 1985). The pomace retained on the sieve is discarded or used as animal feed while the filtrate is fermented for 2-3days to yield *ogi,* which is sour, starchy sediment with water on top. This supernatant is called “omi-eko”, “omikan” or *omidun*.

Microbiological and nutritional studies by Akinrele (1970) showed that four major groups of organisms were responsible for fermentation and nutritional improvement of *ogi* and they include: the Lactic Acid Bacteria (LAB) comprising of *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Acetobacter spp*; aerobic bacteria mainly *Corynebacterium*; the yeasts which are *Saccharomyces cerevisiae* and *Rhodotorula*; and moulds, made up of *Cephalosporium, Fusarium, Aspergillus* and *Penicillium species*. *L. plantarum* was the predominant organism responsible for lactic acid production in the fermentation of *ogi*. *Corynebacterium* was reported to hydrolyse corn starch to organic acids, *Aerobacter cloacae* was capable of increasing the riboflavin and niacin content of the mash while *S. cerevisiae* and *C. mycoderma* were found to contribute to flavour development (Odunfa,1985).

Omidun has been used traditionally to prepare several medicinal herbs in Nigeria. It is used to soak bark of root of some plants to treat fever and malaria. It is also popularly used as solvent for herbal extraction (Aibinu *et al*., 2007).

Earlier reports have shown the microbiological, nutritional and therapeutic values of *ogi* but there is paucity of information on the microbiological and therapeutic effect of the microorganisms in *omidun*. The presence of microorganisms and their attendant vitamins and soluble nitrogenous compounds present in *ogi* cannot be ruled out in *omidun.* Hence this research to isolate and identify microorganisms associated with *omidun.*

2. Materials and Methods

2.1 Collection of Samples

Thirty (30) samples of *omidun* prepared by soaking and fermentation of maize grains in cold water for 28 - 72 h, wet milled, sieved and left to sediment for 72 h, were collected from traditional processors of *ogi* in Obantoko, Abeokuta, Nigeria. Fifteen samples were from *ogi* prepared from white maize grains while the remaining fifteen was from yellow maize grains. The *omidun* samples were collected aseptically in sterile screw cap bottles, labelled and transported to the laboratory for analyses.

2.2 pH and Total Titratable Acidity (TTA)

The pH of each sample was determined using Extech model 30451 pH meter with a reference glass electrode and recorded. The TTA was determined by titrating 10 ml of *omidun* samples (in triplicates) against 0.1M NaOH. The TTA values were calculated and recorded.

2.3 Identification of Microorganisms

Ten-fold serial dilutions up to 10^{-5} of the *omidun* samples were prepared. Lactic Acid Bacteria were isolated by pour plate method on de Man, Rogosa and Sharpè (MRS) medium (Oxoid CM 359). The plates were incubated at 37ºC for 48 h under anaerobic conditions using Gas Pak (H_2+Co_2) anaerobic systems. Colonies from the incubated plates were subcultured on fresh MRS agar plates by streaking to isolate pure culture of the organisms. All pure isolates were stored on Nutrient Agar slants for further characterization and identification. Identification of bacteria was based on cultural, morphological and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

2.4 Yeast and Moulds

Yeasts were isolated on Sabouraud Dextrose Agar (SDA) while moulds were isolated on SDA complemented with 60 µg/ml chloramphenicol (to inhibit bacterial growth). Then the plates were incubated at 25-30ºC for 48-72 hours. Colonies were subcultured on fresh SDA agar plates for purification. Identification of the isolates was based on cultural, morphological and biochemical characteristics (Barnett and Hunter, 1987; Pitt and Hocking, 1997; Samson *et al*., 2004).

3. Results

3.1 pH and Total Titratable Acidity (TTA) of *omidun*

The pH ranged from 2.9 to 4.2 (mean = 3.6) in *omidun* obtained from white maize while the TTA ranged from 0.5 to 1.8%. There was a significant difference ($p<0.05$) in the pH obtained in the individual samples of *omidun* obtained from white maize (Table 1). *Omidun* from yellow maize had pH ranging from 3.4 to 4.5 (mean = 4.0) with the TTA ranging from 0.2 to 1.8% (Table 2). The pH and TTA in the *omidun* samples from yellow maize was significantly different ($p<0.05$) among all the samples. The mean pH of *omidun* from yellow maize was significantly higher ($p<0.05$) those from white maize. Similarly, the mean TTA was significantly ($p<0.05$) dependent on the type of maize used to produce the *omidun.*

3.2 Microorganisms isolated from *omidun*

Microorganisms isolated from *omidun* include bacteria, yeasts and moulds. The main occurring bacterium isolated from all *omidun* samples was *L. plantarum*; the yeasts strains isolated include *Candida albicans C. parasilopsis, C. pseudotropicalis, C. tropicalis, S. cerevisiae;* while *Aspergillus fumigatus* was the only mould isolated (Tables 3 and 4). The percentage incidence of the isolates is shown in Figure 1. *Omidun* from white maize had higher occurrence of *L. plantarum*. The occurrence of *A. fumigatus* and other yeasts were higher in the *omidun* obtained from yellow maize compared with the *omidun* from white maize.

Table 1: Mean pH and Total Titratable Acidity (TTA) of *omidun* obtained from white maize

Sample Code	pH	TTA (%)
1	3.7±1.4d	1.5±0.8cd
2	2.9±1.4h	0.5±0.2i
3	3.1±0.1g	1.7±1.0b
4	3.9±1.3c	0.8±0.2gh
5	3.3±0.4f	1.8±1.1b
6	4.0±0.2c	1.7±0.2bc
7	3.3±0.5f	0.8±0.4h
8	4.2±0.1a	1.5±0.6de
9	4.2±0.3a	1.4±0.2ef
10	3.4±0.5f	1.3±0.4f
11	3.5±0.7e	0.9±0.0g
12	3.1±0.2g	2.4±0.5a
13	3.0±0.7h	1.4±0.1de
14	3.6±0.5d	0.8±0.1h
15	4.1±0.2b	0.5±0.2i
Mean	**3.6±0.4**	**1.3±0.5**

Mean values within a column with the same letter are not significantly different (p>0.05)

Table 2: Mean pH and Total Titratable Acidity (TTA) of *omidun* obtained from yellow maize

Sample Code	pH	TTA (%)
16	3.4±0.6f	1.0±0.1bc
17	3.6±0.2f	1.5±0.8cd
18	3.6±0.2f	1.8±1.4a
19	3.6±0.3f	1.0±0.0bc
20	4.0±0.7cde	0.3±0.1g
21	4.9±1.3a	0.6±0.0e
22	4.0±0.3cde	0.5±0.0ef
23	4.2±0.3bcd	0.3±0.0g
24	4.1±0.2cd	0.4±0.1fg
25	4.3±0.4bc	0.2±0.1g
26	3.6±0.8f	0.5±0.1f
27	3.7±0.2ef	0.8±0.3d
28	4.2±0.9bcd	0.9±0.1cd
29	4.0±0.1de	0.3±1.2g
30	4.5±0.1b	1.1±0.1b
Mean	**4.0±0.4**	**0.7±0.4**

Mean values within a column with the same letter are not significantly different (p>0.05)

Table 3: Microorganisms isolated from *omidun* samples obtained from white maize

Sample code	Bacteria	Yeasts	Moulds
1	*Lactobacillus plantarum*	*Candida albicans, C. parasilopsis, C. pseudotropicalis*	-
2	-	*C. albicans, C. parasilopsis, C. pseudotropicalis, S. cerevisiae*	*Aspergillus fumigatus*
3	*L. plantarum*	*C. albicans, C. parasilopsis,*	-
4	-	*Candida albicans, C. parasilopsis, S. cerevisiae*	-
5	-	*Candida albicans, C. pseudotropicalis*	-
6	-	*Candida albicans, C. parasilopsis, C. pseudotropicalis*	-
7	*L. plantarum*	*Candida albicans, S. cerevisiae*	-
8	-	*Candida albicans, C. tropicalis*	-
9	*L. plantarum*	*Candida albicans, C. parasilopsis, S. cerevisiae*	-
10	-	*Candida albicans, C. tropicalis*	-
11	-	*Candida albicans, C. pseudotropicalis*	-
12	-	*Candida albicans, S. cerevisiae*	-
13	*L. plantarum*	*Candida albicans, C. parasilopsis,*	-
14	-	*Candida albicans, C. tropicalis, C. pseudotropicalis*	-
15		*Candida albicans, C. parasilopsis, S. cerevisiae*	-

Table 4: Microorganisms isolated from *omidun* samples obtained from yellow maize

Sample code	Bacteria	Yeasts	Moulds
16	-	*Candida albicans, Saccharomyces cerevisiae*	-
17	*L. plantarum*	*Candida albicans, C. tropicalis*	-
18	-	*Candida albicans, C. pseudotropicalis*	-
19	-	*Candida albicans, S. cerevisiae*	-
20	-	*Candida albicans, S. cerevisiae*	-
21	*L. plantarum*	*Candida albicans, C. parasilopsis, C. tropicalis*	*Aspergillus fumigatus*
22	-	*Candida albicans, C. pseudotropicalis*	-
23	*L. plantarum*	*Candida albicans, C. tropicalis*	-
24	-	*Candida albicans, C. parasilopsis, C. tropicalis*	-
25	-	*Candida albicans, C. parasilopsis, S. cerevisiae*	-
26	-	*Candida albicans, C. tropicalis S. cerevisiae*	-
27	-	*Candida albicans, C. parasilopsis,*	-
28	*L. plantarum*	*Candida albicans, C. tropicalis*	*Aspergillus fumigatus*
29	-	*Candida albicans, C. parasilopsis,*	-
30	*L. plantarum*	*Candida albicans, S. cerevisiae*	-

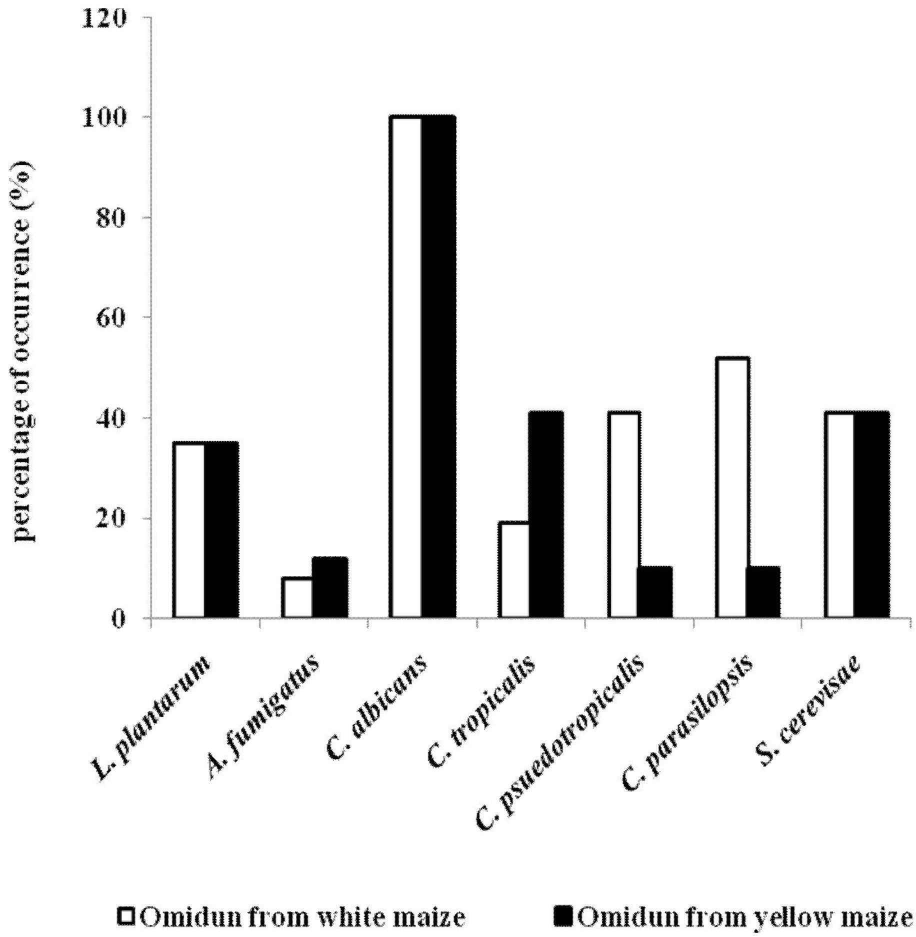

Figure 1: Percentage occurrence of microbial isolates from *omidun* obtained from White maize and yellow maize

4. Discussion

This study has shown that both *omidun* from white maize and yellow maize showed varying pH and TTA. The varying pH and TTA may be attributed to the various types of microorganisms present in the fermented *omidun* samples (Odunfa and Adeyele, 1985). This result is similar to the work of Omemu *et al*. (2007a) who found pH during the souring period of *ogi* ranging from 3.69 to 4.62. The low pH observed in the *omidun* samples studied is also similar to the report of Adebolu *et al.,* 2007 who reported that the low pH confirm the presence of organic acids like lactic acid in *omidun.* Microorganisms isolated in *omidun* samples include *Lactobacillus plantarum, Candida albicans, C. tropicalis, C. pseudotropicalis, C. parasilopsis, Sacchcaromyces cerevisiae* and *Aspergillus fumigatus*. The presence of these microorganisms in *omidun* may be as a result of the fact that they are common microorganisms associated with the natural fermentation of *ogi*. Johansson *et al.* (1995) reported that lactobacilli are the key actors in the fermentation of *ogi*. The importance of *L. plantarum* in the fermentation of *ogi* has also been observed by Odunfa and Adeyele (1985) and Oyarekua *et al.* (2008). *Omidun* from white maize had higher occurrence of *L. plantarum*. The high occurrence of more types of yeasts may be attributed to the high acidity of the *omidun* (Omemu *et al.,* 2007a).

The yeasts, *Candida albicans, C. tropicalis, C. pseudotropicalis, C. parasilopsis and Saccharomyces cerevisiae* observed in this work have been reported to be involved in the fermentation of *ogi* (Omemu *et al.,* 2007a,b), fermentation of other cereals (Jespersen *et al.,* 1994) and in *fufu* production (Oyewole, 2001). These yeasts have also been reported to impact flavour and aroma to fermented foods (Hamad *et al*., 1992). Lactobacilli have been reported to be responsible for the production of lactic acid and other organic acids (Odunfa, 1985), bacteriocins (Garneau *et al*., 2002; Elegado *et al.,* 2004) and other metabolites which have proven to have antimicrobial effects (Afolabi *et al*., 2008). Several studies have been done on bacteriocins because of its potential as biopreservatives in foods (Ross *et al*., 2002).

Aspergillus fumigatus was the only mould isolated from *omidun* and this may be due to the presence of lactic acid bacteria. Teniola and Odunfa (2001) had reported that the presence of high number of Lactic Acid Bacteria in the fermentation of maize for *ogi* production may contribute to the elimination of moulds. *Rhizopus nigricans*, *Aspergillus* and *Penicillium* spp have been reported to be involved in the spoilage of stored wet *ogi Aspergillus fumigatus* (Onyekwere *et al*., 1989). The mould isolated from omidun in this study, *A. fumigatus*, is not only associated with food spoilage as it has also been reported to produce fumigacin, which has antimicrobial activities and is been used as a health care product **(**Waksman *et al.*, 1943). The presence of *A. fumigatus* might be probably from the contact the maize grains have with the soil during the process of drying and milling.

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