Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (2): 141-148, 2007
ISSN 1816-4935

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

[1]A.M. Omemu, [1]M.O. Bankole, [2]O.B. Oyewole and [1]A.K. Akintokun
[1]Department of Microbiology, College of Natural Sciences, University of Agriculture, Abeokuta (UNAAB), P.M.B. 2240, Nigeria
[2]Association of African Universities, African Universities House, Airport Residential Area, P.O. Box AN 5744, Accra, Ghana

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 nigrican* and *Penicillium* sp. while the yeasts are *Saccharomyces cerevisiae* (strain 1), *Candida krusei* (strain 1), *C. 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; Kingamkono *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 Adeyele, 1985;

Corresponding Author: A.M. Omemu, P.O. Box 1164, Sapon, Abeokuta, Ogam State, Nigeria
Tel: 234 08023218008

Odunfa *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 (Onyekwere *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. A total of 20 days storage period was observed. The parameters used to monitor spoilage were as described by Teniola and Odunfa (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} chlortetracycline 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 use 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 (H_2S) and ammonia were noted until the 16th day of storage after which there were declines. More hydrogen sulphide (H_2S) was produced during storage than ammonia (NH_3). 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 ($p \leq 0.05$). 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).

Yeasts 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 1: Changes in the parameters used to monitor spoilage during the storage of *ogi*

Storage days	pH±SE*	TTA (%)±SE*	H_2S±SE*	Ammonia±SE*	Reducing sugars±SE*	Total acceptability±SE*
1	4.32±0.4	0.02±0.0	0±0.0	0±0.0	3.2±0.6	9±0.3
2	3.65±0.3	0.35±0.01	10±0.7	20±1.9	2.8±0.4	8±0.5
4	3.10±0.4	0.52±0.02	40±1.2	40± 3.4	2.6±0.5	7±0.6
6	3.14±0.2	0.44±0.01	62±2.4	60±3.8	1.5±0.6	7±0.4
10	3.18±0.2	0.32±0.01	100±6.7	85±2.9	1.2±0.6	5±0.4
14	3.20±0.3	0.25±0.02	220±5.4	100±5.6	1.0±0.4	2±0.1
16	3.30±0.5	0.23±0.01	250±4.6	150±4.5	0.8±0.1	0±0.0
18	3.66±0.6	0.21±0.02	240±4.8	120±4.9	0.6±0.05	0±0.0
20	3.71±0.4	0.19±0.02	230±3.9	100±3.4	0.4±0.02	0±0.0

*Mean of triplicate determinations±standard error

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

	Steeping period		Souring period	
Time (h)	Yeast population*	Mould population*	Yeast population*	Mould population*
M**	1.77±0.38	6.86±1.68	ND	ND
0	2.90±1.90	6.81±1.59	4.65±1.01	0.00±0.00
12	4.73±1.00	3.72±0.09	5.32±1.04	0.00±0.00
24	5.60±1.49	0.00±0.00	6.92±2.00	0.00±0.00
36	6.22±1.33	0.00±0.00	7.34±0.48	0.00±0.00
48	6.59±2.17	0.00±0.00	7.92±2.42	0.00±0.00

*Mean of triplicate determinations (log cfu g^{-1})±standard error **Maize grains

Yeasts 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 ($p\leq 0.05$). 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 *Candida vini*, *Candida krusei*, *Candida tropicalis*, *Saccharomyces cerevisiae*, *Geotrichum candidum*, *G. fermentans* and *Rhodotorula graminis*. The moulds isolated were *Aspergillus niger*, *A. flavus*, *Fussarrium subglutinans*, *Rhizopus nigricans* and *Penicilium 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 *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 *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 *ogi* sample. Changes in yeasts and moulds populations in the water and the *ogi* samples over time were highly significant ($p\leq 0.05$). 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 *ogi* at room temperature are presented in Fig. 1 and 2, respectively.

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

At the beginning of storage, *S. cerevisiae* (strain 1), *C. krusei* (strain 1), *C. krusei* (strain 2), *C. tropicalis*, *C. vini* (strain 1) and *G. candidum* were obtained. However, by the 10th day of storage, *S. cerevisiae* (strain 1) was not isolated again. *Candida vini* (strain 2) was observed as from the 4th day of storage and remained through out the storage period. Similarly, *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 *ogi*

	Corn steep liquor		*ogi*	
Storage days	Yeast population*	Mould population*	Yeast population*	Mould population*
1	4.62±1.05	0.00±0.00	3.51±0.25	0.00±0.00
2	6.92±1.55	0.00±0.00	3.63±0.15	0.00±0.00
4	7.93±5.50	0.00±0.00	4.80±3.25	0.00±0.00
6	7.96±1.77	0.00±0.00	5.71±3.37	0.00±0.00
8	8.81±2.29	0.00±0.00	6.32±1.00	0.00±0.00
10	8.85±2.36	2.51±2.00	6.80±2.00	0.00±0.00
12	8.96±2.00	3.82±0.20	6.93±1.00	$<10^2$
14	8.87±2.00	4.46±1.52	7.33±1.00	2.08±0.21
16	7.87±2.00	5.14±0.60	6.87±0.20	3.81±0.45
18	6.62±2.20	6.82±2.20	6.54±2.00	4.51±2.00
20	4.72±3.00	8.80±2.00	4.65±1.40	6.74±2.00

*Mean of triplicate determinations (log cfu g^{-1})±standard error

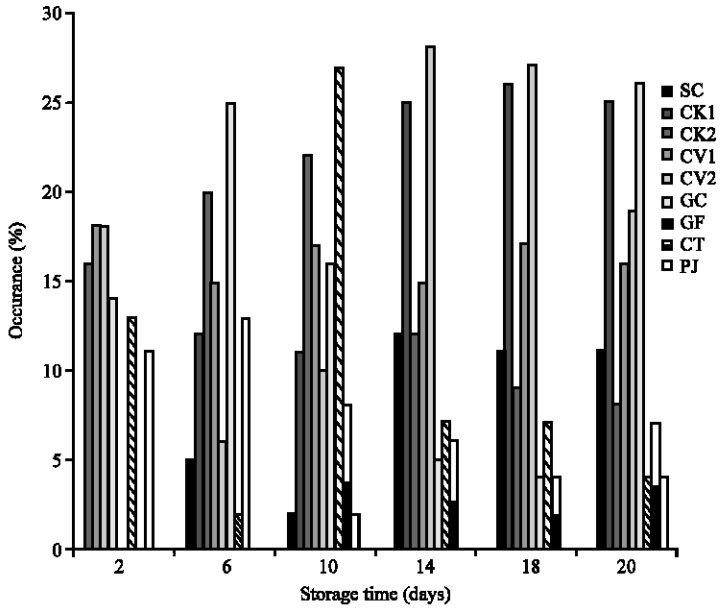

Fig. 1: Succession and frequency distribution of yeasts isolates during the storage of *ogi*, SC-*S. cerevisiae*; CK1-*C. krusei* (strain 1), *C. krusei* (strain 2); CVI-*C. vini* (strain 1); CV2-*C. vini* (strain 2); GC-*G. candidum*; GF-*G. fermentans*; CT-*C. tropicalis*; PJ-*P. japonicum*

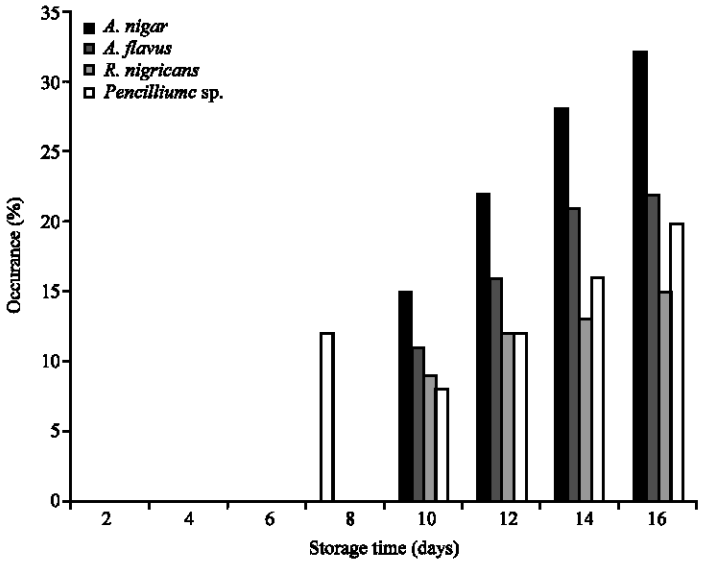

Fig. 2: Succession and frequency distribution of molds isolates during the storage of *ogi*

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 nigrican* 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 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; Rohm *et al*., 1992). Yeasts are common contaminants of fruit and some dairy products like cream, butter and cheese (Deak and Beuchat, 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 (Nuraida *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), trichothecenes 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 (Pfohl-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

Adeniji, A.O. and N.N. Potter, 1978. Properties of *ogi* powders made from normal, fortified and opaque-2 corn. J. Food Sci., 43: 1571-1574.

Akinrele, I.A., 1970. Fermentation studies on maize during the preparation of a traditional African starch-cake food. J. Sci. Agric., 21: 619-625.

Aremu, C.Y., 1993. Nutrient composition of corn *ogi* prepared by a slightly modified traditional technique. Food Chem., 46: 231-233.

Barnett, J.A., R.W. Payne and D. Yarrow, 1990. YEASTS: Characteristics and Identification. 2nd Edn., Cambridge Univ. Press, pp: 1002.

Deak, T. and L.R. Beuchat, 1993. Yeasts associated with fruit juices concentrates. J. Food Protein, 56: 777-782.

El-Gendy, M. and E.H. Marth, 1980. growth of toxigenic and non toxigenic aspergilli and penicillia at different temperatures and in the presence of lactic acid bacteria. Arch. Lebensmittelhyg, 31: 189-220.

Fung, D.Y.C. and C. Liang, 1990. Critical review of isolation, detection and identification of yeasts from meat products. Cri. Rev. Food Sci. Nutr., 29: 341-379.

Jespersen, L., M. Halm, K. Kpodo and M. Jacobsen, 1994. Significance of yeasts and moulds occurring in maize dough fermentation for *Kenkey* production. Int. J. Food Microbiol., 24: 239-248.

Jonsyn, F.E., 1989. Fungi associated with selected fermented foods in Sierria Leone. MIRCEN J. Applied Microbiol. Biotechnol., 5: 457-462.

Kingamkono, R., E. Sjogren, U. Svanberg and B. Kaijser, 1995. Inhibition of different strains of enteropathogens in a lacticfermenting cereal gruel. World J. Microbiol. Biotechnol., 11: 299-303.

Lorri, W. and U. Svanberg, 1993. Lactic-fermented cereal gruels with improved *in vitro* protein digestibility. Int. J. Food Sci. Nutr., 44: 29-36.

Mensah, P., 1997. Fermentation-the key to food safety assurance in Africa? Food Control, 8: 271-278.

Nuraida, L., M.C. Wacher and J.D. Owens, 1995. Microbiology of *Pozol*, a Mexican fermented maize dough. World J. Microbiol. Biotechnol., 11: 567-571.

Odunfa, S.A., 1985. African fermented foods. In: Microbiology of Fermented Foods. B.J.B. Wood, Elsevier Science, London and New York, pp: 155-199.

Odunfa, S.A. and S. Adeyele, 1985. Microbiological changes during the traditional production of *ogi*-baba, a West African fermented sorghum gruel. J. Cereal Sci., 3: 173-180.

Odunfa, S.A., S.A. Adeniran, O.D. Teniola and J. Nordstrom, 2001. Evaluation of lysine and methionine production in some lactobacilli and yeasts from *ogi*. Int. J. Food Microbiol., 63: 159-163.

Olasupo, N.A., D.K. Olukoya and S.A. Odunfa, 1997. Assessment of a bacteriocin-producing Lactobacillus strain in the control of spoilage of a cereal-based African fermented food. *Folia* Microbiol., 42: 31-34.

Onyekwere, O.O., I.A. Akinrele and O.A. Koleoso, 1989. Industrialization of *ogi* fermentation. In: Industrialization of Indigenous Fermented Foods, 33: 329-362.

Oyewole, O.B., 1997. Lactic fermented foods in Africa and their benefits. Food Control, 8: 289-297.

Pfohl-Leszkowicz, A., T. Petkova-Bocharova, I.N. Chernozemsky and M. Castegnaro, 2002. Balkan endemic nephropathy and associated urinary tract tumours: A review on aetiological causes and the potential role of mycotoxins. Food Add. Contam., 19: 282-302.

Pitt, J.I. and A.D. Hocking, 1999. Fungi and Food Spoilage. Gaithersburg, Maryland: Aspen Publishers Inc.

Restuccia, C., C. Randazzo and C. Caggia, 2006. Influence of packaging on spoilage yeast population in minimally processed orange slices. Int. J. Food Microbiol., 109: 146-150.

Rheeder, J.P., W.F.O. Marasas, P.G. Thiel, E.W. Sydenham, G.S. Shephard and Van D.J. Schalkwyk, 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology, 82: 353-357.

Rohm, H., F. Eliskases-Lechner and M. Brau¨er, 1992. Diversity of yeasts in selected dairy products. J. Applied Bacteriol., 72: 370-376.

Tchango, J., P. Tailliez, P. EB, T. Njine and J.P. Hornez, 1997. Heat resistance of the spoilage yeasts *Candida pelliculosa* and Kloeckera apis and pasterurization values for some tropical fruit juices and nectars. Food Microbiol., 14: 93-99.

Teniola, O.D. and S.A. Odunfa, 2001. The effects of processing methods on the level of ysine, methionine and the general acceptability of *ogi* processed using starter cultures. Int. J. Food Microbiol., 63: 1-9.

Teniola, O.D. and S.A. Odunfa, 2002. Microbial assessment and quality evaluation of *ogi* during spoilage. World J. Microbiol. Technol., 18: 731-737.

Vainio, H., E. Hesseltine and J. Wilbourn, 1993. Report on an IARC working group meeting on some naturally occurring substances. Int. J. Cancer, 53: 535-537.

Viljoen, B.C., 2001. The interaction between yeasts and bacteria in dairy environments. Int. J. Food Microbiol., 69: 37-44.