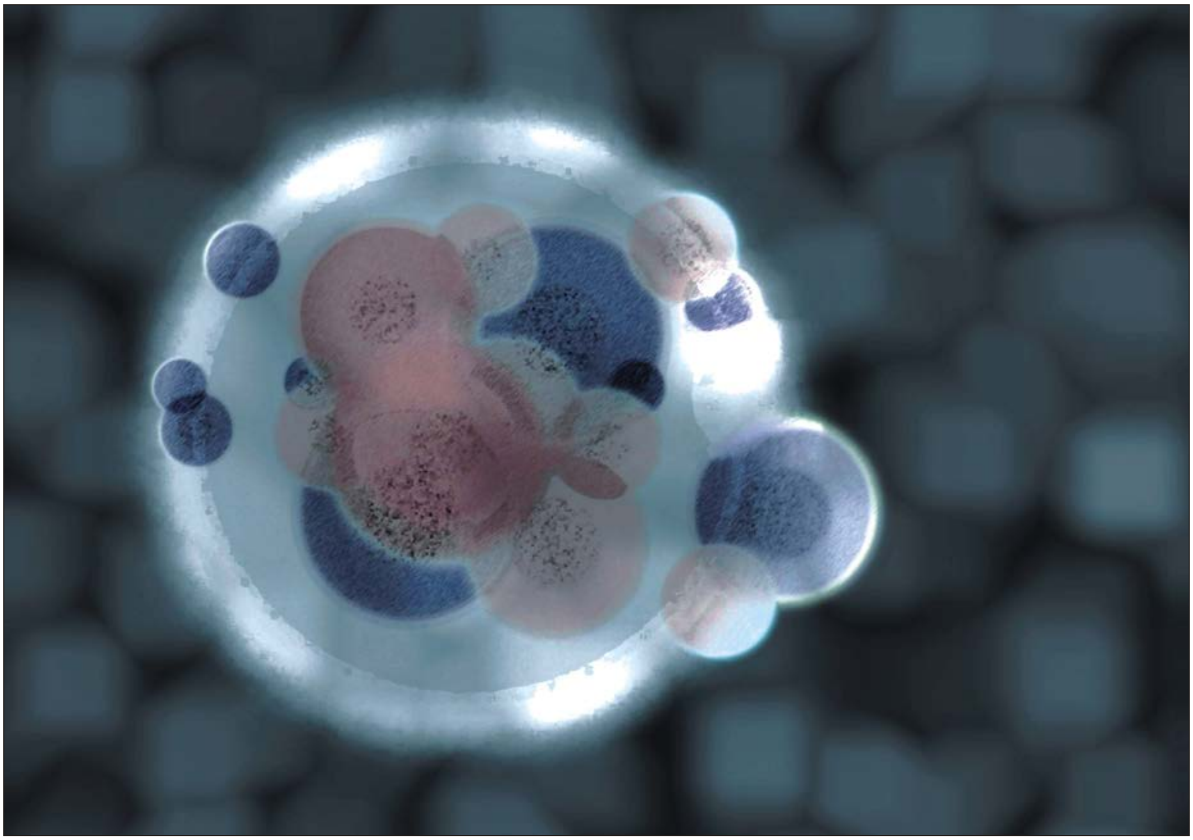

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Spoilage of Some Stored Fermented Foods in South West Nigeria

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Abstract: The signs of deterioration and spoilage symptoms of the following locally fermented foods; *Ogi, Eko, Eba* and *Fufu* stored at refrigeration (5±2°C) and room (28±2°C) temperatures were studied. The foods stored at refrigeration temperature stayed relatively longer and kept better, between 16-71 days *Eko* and *Pap* {16 days}; {71 days}, respectively before total rejection. While the same products stored at room temperature (28±2°C) stayed for only five days. Species of *Aspergillus, Pennicillium, Curvularia, Neurospora, Mucor, Fusarium* and *Geotrichum* were implicated in the spoilage of the food products. *Aspergillus* and *Penicillium* sp. were the most common isolates obtained from all the food products, but only *Ogi* and *Pap* recorded the incidence of *Geotrichum* sp. Fresh *Ogi* exhibited the highest concentration of most of the mineral elements (e.g., iron 38.66 mg; magnesium 5.93 mg; potassium 75.19 mg per 100 g) investigated. However the low values obtained in the spoilt samples were due to the extent of metabolic activities of the spoilage organisms where iron, for example, was reduced by 56.8 and 94.9% in *Eba* and *Eko,* respectively. All the isolates grew well on the food extract agar and broth media with *Neurospora* sp. exhibiting the most appreciable growth on *Eko* extract agar (81 mm) and broth (89 mg).

Key words: Fermented foods, spoilage system, fungi

INTRODUCTION

Fermented foods constitute a significant component of the African diet for ages. Many fermented foods are consumed as the main course while few are offered as beverages or highly priced food condiments (Odunfa, 1985). Those that serve as main meals and beverages are usually from fermentation of carbohydrate-rich raw materials most of which have low protein and vitamin contents. Some of these include mahewu obtained from maize; *Kaffir* beer from sorghum; *Gari* and *Fufu* from cassava.

Fermented food products are more palatable and do not easily get spoilt because the by-product(s) of fermentation which also include organic acids, hydrogen peroxide, diacetyl and bacteriocin serve as preservatives against spoilage organisms (Gibbs, 1987; Klaenhammer, 1988; Garriga *et al*., 1993) apart from imparting their desired flavour and aroma. Spoilage of food may be seen as any change that occurs in a food, making it unacceptable for human consumption (Brackett, 1997). Most food spoilage are due to the activities of bacteria, molds and yeasts, which alter the smell, taste, colour, texture or chemical composition of the food significantly and render it inedible (William and Shaw, 1992).

The chemical properties of the food determine how satisfactory it will be as a culture medium for microbial growth. Carbohydrate-rich foods are often spoilt by fungi especially species of *Rhizopus, Mucor* and *Penicillium* which grow on starchy foods if kept in humid condition (William and Shaw, 1992).

Information on the storage conditions and the organisms involved in the spoilage of fermented foods commonly consumed in the south west of Nigeria are scanty. This study therefore reports on the fungi associated with the spoilage of some Nigerian fermented food products stored at both the refrigeration and room temperatures.

MATERIALS AND METHODS

Sources of samples: Each of the fermented plant food products; *Eba, Ogi, Pap* and *Fufu* was obtained from a local market in Akure, Ondo State, wrapped in transparent polythene bags (ca. 0.16 mm thick). *Eko* was however offered for sale, wrapped in leaves of *Thaunatococcus daniella*. All these plant food products are obtained through fermentative acidification by lactic acid bacteria (Olukoya *et al*., 1993).

Preparation of fermented: *Ogi* is fermented starch cake processed from maize or sorghum (Onyekwere *et al*., 1989; Asiedu, 1989). The preparation of *Pap* was done by quickly mixing and stirring the *Ogi* slurry with boiling

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water in a container until a thick gel is obtained. The product was allowed to cool to room temperature, covered and stored. Conventionally, both *Ogi* and pap are usually covered with clean water daily or occasionally during storage. *Eko* preparation goes a step further, with continuous stirring on fire (heat) until the water content was greatly reduced and a dough-like product obtained. This was later packaged and stored.

Eba is a pasty dough-like food prepared by mixing gari, a product of fermented grated cassava tubers (Sanni, 1991), with hot water to obtain a semi-solid food. *Fufu* is a product of mashed and sieved cassava root, cooked in hot water to form a thick dough (Adegoke and Babalola, 1989). All the food samples were stored at room (28±2°C) and refrigeration (5±2°C).

Chemical analysis: Fresh and deteriorated samples were analysed separately for moisture, crude fibre, protein, ash and fat contents, pH and titrable acidity as described by Pearson *et al.* (1981) and AOAC (1990). The carbohydrate content was determined by difference. The mineral nutrients (potassium, calcium, sodium, magnesium and iron) were determined using the Atomic Absorption Spectrophotometer (AAS-Bucks 210) as described by Pearson *et al.* (1981), Pearson (1982) and Adeyeye (1997).

Physical examination and microbiological analyses: Food samples stored at refrigeration and room temperatures were observed for change in colour, texture, firmness, odour and appearance of molds and slime. Portions of samples showing signs of spoilage were taken and inoculated directly on malt extract agar. The fungi isolates were observed under the microscope and identified as described by Aderiye (1984) and Barnett (1960). Furthermore, each of these isolates was reinoculated on fresh substrates to confirm which isolate caused the different signs of spoilage.

Preparation of sample extracts: Ten grams of each fresh food sample was suspended in 90 mL-distilled water and homogenized. The solution was heated at 40-50°C for about 10 min for nutrients to dissolve into solution. One gram of dextrose (Analar) was added to each sample extract and the solution standardized with lactic acid to a pH of between 4.0-4.5 to inhibit bacterial growth. The solution was then dispensed after filtration into tubes and sterilized in the autoclave at 1.05 kg cm^{-2} and 121°C for 15 min. The tubes were then inoculated. The un-inoculated malt extract agar plates were used as control. All experiment were carried out in triplicate and repeated twice.

RESULTS AND DISCUSSION

Spoilage symptoms: The signs of deterioration and spoilage symptoms of some locally fermented foodstuffs stored at refrigeration (5±2°C) and room (28±2°C) temperatures are reported in Table 1. Generally, the foods stored at refrigeration temperature stayed relatively longer and kept better than those stored at room temperature. Most of the spoilage organisms in the tropics are mesophilic (Brackett, 1997) and with low temperature (5±2°C), the physiological and metabolic activities of such organisms are drastically reduced. *Eko* wrapped in the leaves of *Thaumatococcus daniella* stayed for about 3 days at 5°C before a yellowish discolouration of the gel-like food product appeared. Meanwhile, after 2 days of storage at 30°C some slimy-to-touch substances were noticed on the same food product when unwrapped.

Ogi and *Fufu* kept better and retained most of their palatability attributes for about 47 and 71 days, respectively at refrigeration (5±2°C) temperature, after which they were found very unacceptable for further processing and/or consumption. It is however interesting to note that these food products were unacceptable after noticing molds growth within 6-8 days of storage (Table 1). Both *Ogi* and *Pap*, when submerged in water during storage, showed a creamish and cloudy appearance on the liquor surface. This thin filmy substance was also slimy to touch. At a later stage, the *creamish* growth changed to grey. Sliminess in both cases was attributed to the presence of *Geotrichum* sp. and some bacterial species which are not to be identified.

Table 1: Spoilage symptoms/signs of deterioration of fermented foods

	Spoilage symptoms/signs of deterioration (days)					
Fermented foods (Storage conditions)	Storage temp.	Sliminess	Discolouration	Mold growth	Off odour	Rejection
Ogi (submerged in water)	5°C	20[a]	29[b]	39	29	47
	28°C	14[a]	12	19	15	22
Pap (submerged in water)	5°C	10[a]	8	14	6	16
	28°C	4[a]	4[b]	3	2	5
Eko (Wrapped in leaf)	5°C	12	3	9	8	21
	28°C	2	3	3	4	5
Eba (Wrapped in cellophane)	5°C	14	3	21	15	25
	28°C	3	2	4	2	6
Fufu (Wrapped in cellophane)	5°C	ND	29	65	7	71
	28°C	12	6	9	ND	13

[a]Also shows cloudy, creamish colonies on surface of liquor, [b]Creamish colonial growth on surface of liquor changed to grey,
ND =Sliminess and off-odour were not detected in *Fufu* stored in 5 and 28°C, respectively

Table 2: Chemical constituents of fresh and stored *fermented foods

Chemical component (mg g^{-1})	Ogi Fresh	Ogi Stored	Pap Fresh	Pap Stored	Eko Fresh	Eko Stored	Eba Fresh	Eba Stored	Fufu Fresh	Fufu Stored
Ash	2.41	3.80	1.08	2.01	2.53	3.61	4.42	4.91	3.18	4.82
Fat	73.31	55.16	87.91	45.50	87.41	4.20	75.11	47.90	92.83	58.22
Crude fibre	ND	ND	ND	ND	ND	ND	7.51	6.72	6.01	5.35
Crude protein	37.40	40.72	10.00	29.92	14.31	37.90	1.99	37.80	1.29	4.61
Moisture	486.10	598.00	880.60	902.30	872.00	898.10	738.40	793.80	712.50	787.80
Carbohydrate	419.90	302.30	20.14	20.29	23.75	16.19	174.40	109.50	184.20	99.20
TTA (%)	4.88	5.09	2.63	6.67	2.16	7.20	2.91	8.37	4.14	9.00
pH	3.81	3.49	4.79	3.58	4.40	3.22	4.36	2.95	4.13	2.17

* Food samples were analysed when mold growth occurred., ND: Food samples did not show any traces of fibre

Table 3: Some mineral nutrients of fresh and stored *fermented foods

Nutrient (mg 100 g^{-1})	Ogi Fresh	Ogi Stored	Pap Fresh	Pap Stored	Eko Fresh	Eko Stored	Eba Fresh	Eba Stored	Fufu Fresh	Fufu Stored
Calcium	57.06	35.17	50.75	35.46	49.26	19.88	88.73	10.20	39.10	12.37
Iron	38.66	3.60	21.79	2.63	17.44	0.89	1.69	0.73	1.19	0.12
Magnesium	75.93	42.99	55.82	41.68	46.68	16.03	60.20	22.91	64.48	14.80
Potassium	75.19	41.19	69.45	39.82	56.89	19.08	48.72	14.88	58.11	12.17
Sodium	65.51	41.11	72.74	54.50	66.70	20.49	57.94	22.03	54.83	14.32

• Food samples were analysed when mold growth occurred

The presences of *Geotrichum* sp. confirms earlier reports of Teniola and Odunfa (2002) as one of the dominant organisms involved in the spoilage of fermenting *Ogi* mash. They reported that metabolites produced during fermentation are usually responsible for the changes in the physical and chemical parameters. *Pap* and *Eko* are fermented food products that could perish within a week of storage at room temperature.

In *Fufu* and *Eba*, mould growth was observed earlier than sliminess of the products stored at 28°C However, *Fufu* and *Ogi* kept fairly longer for about 2 and 3 weeks, respectively. Incidentally, these two food products kept better at refrigeration temperature for about 7 weeks and more. All these local food products were obtained through fermentive acidification by lactic acid bacteria (LAB), which occur widely as indigenous contaminants during the natural fermentation of maize and cassava (Olukoya *et al*., 1993). However, LAB has been reported to produce antimicrobial substances such as bacteriocin, hydrogen peroxide and diacetyl especially against spoilage organisms (Teniola and Odunfa, 2002; Garvier and Muriana, 1993; Olasup *et al*., 1994; Olasupo, 1996). These bacteriocins are relatively heat-stable, usually safe for consumption and with promising inhibitory spectra of antimicrobial activities (Olasupo *et al*., 1994). Olukoya *et al*. (1993) reported the presence of plantaricin in *Fufu* obtained from cassava fermented by *Lactobacillus plantarum strain* SDK9. The bacteriocin was found to inhibit the growth of strains of *L. plantarum, L brevis, L. sake* and *Enterococcus faecalis.*

Occurrence: Species of *Aspergillus* and *Penicillium* are the most common isolates obtained from all the food products even at the different storage conditions. *Curvularia* sp. was obtained from *Ogi, Eba* and *Fufu,* while *Neurospora* sp. was found only in *Eba* and *Eko*. All the food products except *Ogi* showed the presence of *Mucor* sp. while *Fusarium* sp. occurred in all food products excepts *Pap*. Only *Ogi* and *Pap* showed the presence of *Geotrichum* sp.

Chemical component of fermented foods: In all cases, there was reduction in the crude fibre, fat, ash and carbohydrate contents of the stored food products (Table 2). Expectedly, the crude protein content of each stored fermented product increased since the spoilage organisms utilized the available nutrients especially carbohydrate and mineral nutrients to build up the microbial mass (Olasupo, 1996). The pH and Titratable Acidity (TTA) of the food products are also shown in Table 2. The acidity (estimated as % lactic acid) of each food sample increased as storage progressed. Therefore, the pH of the substrates decreased following long periods of storage; a situation, which favoured the incidence of more fungal colonies.

Table 3 shows some of the nutritionally important mineral nutrients present in both fresh and spoilt fermented food samples. These elements played significant roles in the biological systems of the fungi isolates. Fresh *Ogi* had the highest concentration of most of the mineral elements (iron 38.66 mg, magnesium 75.93

Table 4: Mycelia mass (mg) of fungi isolates grown on food[a] and malt extract both media

Fungi isolate	Control	Food substrates				
		Ogi	*Pap*	*Eko*	*Eba*	Fufu
Absidia sp.	47	36	54	52	53	57
Aspergillus flavus	51	42	48	49	41	45
A. niger.	50	41	47	48	42	44
Curvularia sp.	38	44	54	59	47	56
Fusarium sp.	41	28	32	31	30	30
Mucor sp.	45	33	49	40	35	42
Neurospora sp.	81	61	84	89	72	68
Rhizopus sp.	49	36	51	48	44	36
R. stolonifer	70	44	51	69	49	45
Penicillium sp.	34	29	58	39	32	30

Note: Readings were taken after 24 h incubation. [a]Extracts of fermented foods were used for the media formulation, *Control-Malt extract broth,

Table 5: Fungal growth (cm) on food substrate[a] and malt extract media

Fungi isolate	Control	Food substrates				
		Ogi	*Pap*	*Eko*	*Eba*	Fufu
Absidia sp.	1.4	2.3	2.6	0.8	1.0	2.8
Aspergillus flavus	0.7	0.3	0.4	0.5	0.1	0.1
A. niger.	0.7	0.3	0.4	0.5	0.4	0.1
Curvularia sp.	0.8	0.9	2.0	2.7	1.4	1.9
Fusarium sp.	0.9	0.3	0.4	0.4	0.4	0.4
Mucor sp.	2.4	1.6	2.5	2.3	2.0	2.2
Neurospora sp.	6.8	4.3	8.0	8.1	6.2	3.2
Rhizopus sp.	2.6	1.5	3.0	2.8	1.6	0.6
R. stolonifer	7.0	2.7	2.5	4.0	3.0	3.0
Penicillium sp.	0.2	0.1	0.2	0.3	0.1	0.1

Note: Readings were obtained after 24 h incubation [a]Extracts of fermented foods were used for the media formulation, *Control-Malt extract agar,

mg and potassium, 75.19 mg), which have been found useful in the replacement of electrolyte balance in diarrhea patients. This agrees with Aderiye and Laleye (2003) where *Ogi* and *Pap* were reported to play significant role in the management of infantile diarrhea cases in southwest Nigeria. The highest values for sodium and calcium are recorded in freshly prepared *Pap* (72.74 mg) and *Eba* (88.73 mg), respectively (Table 3). The high calcium value recorded in *Eba* agrees with the report of Onyekwere *et al.* (1989) for *Gari*, a product of fermented, finely grated cassava tuber.

The low values obtained for these mineral nutrients in the spoilt samples showed the extent of metabolic activities of the spoilage organisms. For example, iron concentration in *Eba* and *Eko* reduced by 56.80 and 94.89%, respectively. This high reduction rate may be attributed to the presence of *Neurospora* sp. on these food products where the fungus grew prominently. Also *Ogi* showed 90.68% reduction in iron value. Iron has been reported to aid cytochrome heme apoenzyme and pigment formation in microbes (Aderiye and Laleye, 2003; Graffin, 1981; Frazier and Westhoff, 1988).

Microbiological assessment of fermented food products for microbial growth: The growth of the different isolates except *Geotrichum* sp. on the extract broth and agar media showed differing ability of these substrates to support the growth of organisms. These are demonstrated by the mycelia mass (Table 4) and the radial growth (Table 5) of the fungi isolates. *Neurospora* sp. had the highest mycelia mass (89 mg) on *Eko* broth after 24 h while *Fusarium* sp. recorded the least weight (28 mg) on *Ogi* (Table 4).

Most of the fungi isolates had better growth on *Pap* and *Eko* than *Ogi*, even though both *Pap* and *Eko* are products of *Ogi*, obtained from fermented maize. This observation may be due to the effect of prolonged heat processing of these products which might have broken down the nutrients and made them more readily available for the use of these organisms. In addition, the potency of the antimicrobial compounds produced during fermentation may also have been reduced during heating. Adeyeye (1997) reported that heat treatment improved the availability of nutrients in the African yam beans vis-à-vis digestibility, which leads to easy denaturing and opening up of the food structures.

Storage of fermented foods at low temperatures affected the shelf life, the incidence of spoilage organisms and the nutritional composition. In some instances, it took between 3 and 5 times the number of days required for *Pap* and *Fufu* stored at 5±2°C to get spoilt. The inhibitory substances (especially lactic acid and some bacteriocins) produced during the ferments of these foods may have caused the delay in the incidence and growth of the spoilage organisms (Olasupo *et al.*, 1994) particularly the fungi. Also the mode of action of diacetyl has shown that it deactivate enzymes from microorganisms, for example, alcohol dehydrogenase and adenylate cyclase in yeasts and glutamate dehydrogenase and Transketolase in pentose cycle (Deacon, 1988). With refrigeration, therefore, most of the microbial activities are kept low and as such bio deterioration was slow.

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