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Assessment of bacterial and fungal spoilage of some Nigerian fermented and unfermented foods

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The study was aimed at evaluating the microbial spoilage of selected Nigerian fermented and unfermented foods. A total of four fermented and unfermented food samples were used for this investigation. Microbial and sensory evaluations of the food products during storage were carried out using standard procedures. During storage, the bacteria counts were observed to range from 2.2 × 10^5 to 4.8 × 10^5 CFU/mL and from 2.5 × 10^3 to 5.0 × 10^4 CFU/mL, for the unfermented and fermented food products, respectively. Similarly, the fungal counts ranged from 1.8 × 10^3 to 2.9 × 10^3 CFU/mL and from 0 to 5.70 × 10^3 CFU/mL for the unfermented and fermented food samples, respectively. *Klebsiella aerogenes*, *Lactobacillus plantarum*, *Leuconostoc* sp., *Micrococcus varians*, *Proteus mirabilis*, *Streptococcus faecalis, Staphylococcus epidermidis*, *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium herbarum*, *Geotrichum candidum*, *Mucor mucedo*, *Neurospora sitophilia* and *Penicillium* sp. bacteria and fungi that were recovered from the food samples during storage. The sensory evaluation of the food products showed the fermented ones being more acceptable to panelists than the unfermented ones. This could indicate that the palatability of the fermented food samples only experienced slight or no changes during storage, when compared to the unfermented ones.

Key words: Fermented food, unfermented food, microbial spoilage.

INTRODUCTION

It is reported that fermented foods constitute about 25% of the foods consumed worldwide. Fermented foods have many advantageous attributes, which include improved nutritional value and safety against pathogens over non-fermented foods. Generally, these foods are normally considered to be safe against foodborne diseases because of the advantage of low pH, which is due to the presence of organic acids produced during fermentation. In Africa, because a majority of the fermented foods are produced at household levels, the issue of hygiene is a major concern (All and Dardir, 2009; Gadaga et al., 2004).

Both fermented and unfermented food products are known to be susceptible to spoilage during storage. Their spoilage during storage is attributed to the presence of microorganisms and extracellular enzymes produced, which breakdown the food product into new substances resulting into changes in their organoleptic properties

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(Fadahunsi et al., 2013). Chance inoculation, activities of spoilage organisms and the humid condition in the Tropics are some of the factors responsible for spoilage of these foods during storage. While starter cultures have been used to ensure uniformity in the composition of some fermented foods, their reduced shelf life still remains a major problem worldwide. The ingestion of products contaminated with these microorganisms could be a potential health threat hence there is the need to control microbial contamination of fermented foods and feeds.

Filamentous moulds and yeast are common spoilage organisms of food products as fermented milk products, cheese, bread, stored crops and feed such as hay and silage (Filtenborg et al., 1996). *Penicillia* and *Aspergilli* species have been reported as spoilage organisms during storage of a wide range of foods where they may produce a number of mycotoxins (Samson et al., 2002). Several authors (Pitt and Hocking, 1999; Magnusson and Schnurer, 2001) have also implicated yeasts such as *Candida parapsilosis, Rhodotorula mucilaginosa, Kluyveromyces marxians and Derbaromyces hansenii* as common spoilage organisms of yoghurt and other fermented food products.

Apart from food spoilage, health hazards from fungal mycotoxins had been well documented. For instance, the large consumption of grains infected with sclerotia of *Claviceps pupurea* caused several thousand deaths in the 10^{th} and 11^{th} century in central Europe (Pohland, 1993). It is estimated that between 5 and 10% of the world's food products is lost due to fungal deterioration (Pitt and Hocking, 1999). The growth of these undesirable fungal species in foods has been a worldwide concern over the years because of its effects on man's health and consequent economic loss due to food storage. During food spoilage, which is a metabolic process, the spoilt food becomes undesirable and unacceptable for human consumption due to changes in sensory characteristics.

Fermented foods such as gari, ogi, fufu and mawe are found all over the world. Fermentation has offered a means of food security in Nigeria; where over 52% of respondents in a previous study in 2003 indicated that about 25% of their monthly income is spent on fermented food products (Aderiye and Laleye, 2003). This paper therefore examines and discusses the desirability of consuming part of spoiled fermented food products as reported by Aderiye and Laleye (2003). It is also aimed at evaluating the types and extent of microbial spoilage of some selected Nigerian fermented and unfermented foods.

MATERIALS AND METHODS

A total of eight different food samples, divided into unfermented (pounded yam, yam, rice and beans) and fermented ('Eba', 'Eko', 'Fufu' and 'Kati') food products were used in this study. The raw food products were purchased from the local market in Ado-Ekiti, Ekiti State in Nigeria. To ensure uniformity and prevent any pre-storage contamination, the purchased raw food products were prepared locally in the laboratory using aseptic techniques. Each of the prepared food samples was transferred into and wrapped with sterile polythene bags (*ca* 0.2 mm thick), while some were left unwrapped. Food samples from 'Eko' and 'Kati' were wrapped in the leaves of *Thaumatoccus daniella*. This was to ensure that the samples were in the same state as they were normally packaged in the locality. Detail description of the food products and their preparation have been described elsewhere (Aderiye and Lalaye, 2003). All the food products were stored under hygienic conditions at refrigeration and ambient temperatures.

Microbiological analyses were carried out using conventional microbiological procedures. The total microbial count on the food sample was determined by the pour plate method using standard microbiological techniques. Nutrient agar and Malt extract agar were used to culture bacteria and fungi, respectively. All bacterial and fungal counts were expressed as colony forming units per milliliter (CFU/mL) and propagules and cells/mL respectively. In all cases, each analysis was carried out in triplicates. The isolation and identification of the bacteria and fungi were carried out as described elsewhere **(**Barnett and Hunter, 1992; Burgess and Sum, 1994; Holt et al., 1994). The occurrence of each isolate in the food samples was determined as a percentage of the total number of food samples and recorded as the frequency of occurrence. The frequency of occurrence of isolates in the food samples was calculated using the following formula:

$$\%\ \text{frequency of occuerence} = \frac{\text{No of microbial occurrence in the food samples}}{\text{Total number of food samples}}$$

Sensory properties such as appearance, colour, odour, taste, texture and general acceptability were also used in an 11-point 'Hedonic' scale to test the acceptability and rejection of the food samples. The detection of spoilage was done through physical examination of the stored food samples. The wrapped food samples were observed daily for changes in appearance, texture, aroma and taste. The day of onset of each defect or sign of spoilage and the types were recorded.

Each of the experiments was carried out in triplicates and conducted twice. All the statistical analyses were carried out using the SPSS computer statistical software. The comparison of means was done using the One-Way Analysis of Variance Test.

RESULTS

The microbial quality of the food samples is shown in Figures 1 and 2. When the food samples were examined within one hour of processing, no microbial contamination was recorded. As shown in Figure 1, in all the food samples, bacterial colonization was observed within 24 h after production. The bacterial counts in the food samples were observed to increase with increase in storage time. At the end of 96 h storage period, the bacterial counts from the unfermented food samples ranged from 2.2×10^5 to 4.8×10^5 CFU/mL, for yam and pounded yam, respectively. In the fermented food samples, the bacterial count was observed to range from 2.5×10^3 to 5.0×10^4 CFU/mL, for 'Kati' and 'Eba', respectively (Figure 1). In all cases, the bacterial counts in the unfermented food samples were significantly higher than those in the fermented food samples ($P \leq 0.05$). Even after 96 h storage, 'Eba' still

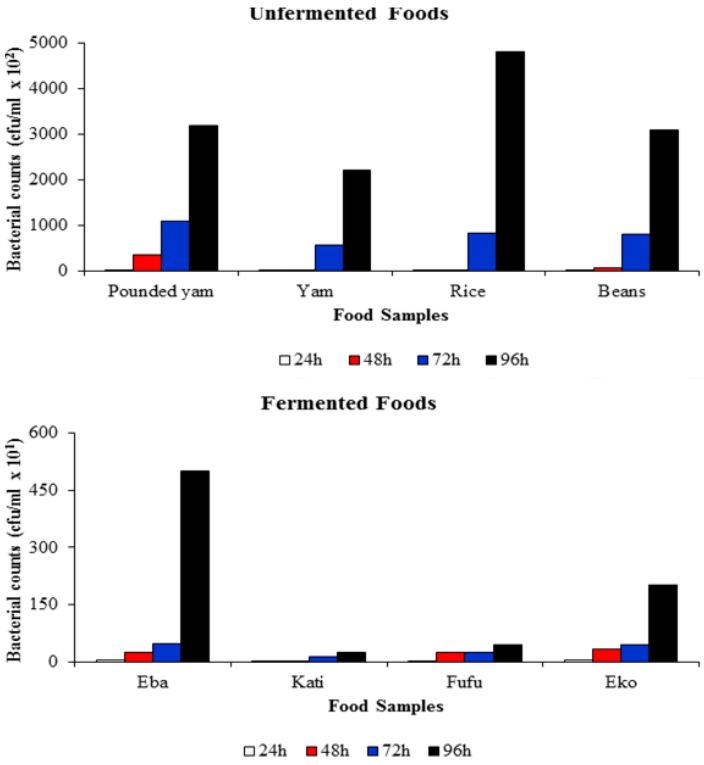

Figure 1. Average bacterial counts of the food samples during the period of storage.

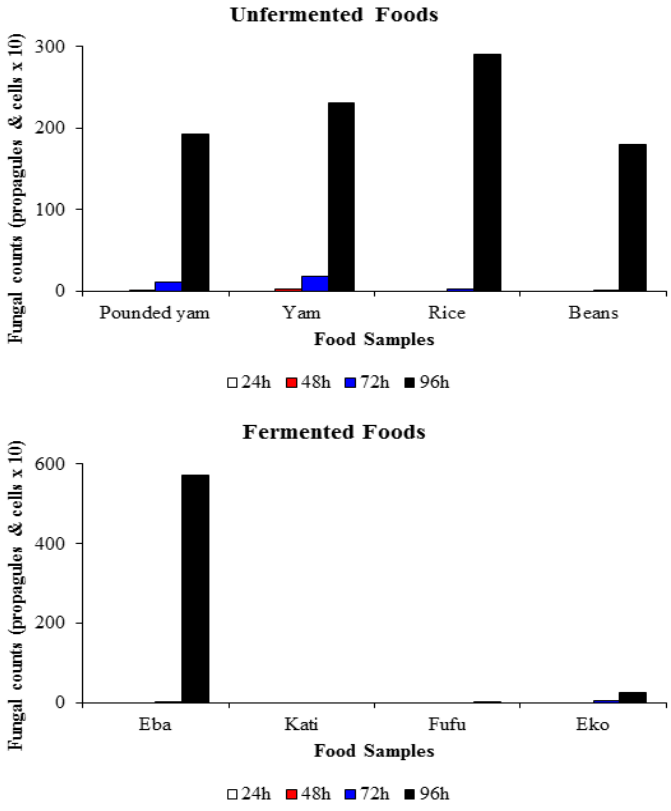

Figure 2. Average fungal counts of the food samples during the period of storage.

Table 1. Occurrence and distribution of bacteria in the food samples.

Food sample	*Kleb.*	*Lact.*	*Leuc.*	*Micr.*	*Prot.*	*Strep.*	*Staph.*
Pounded yam	+	-	-	+	+	+	+
White rice	+	-	-	+	+	-	+
Beans	-	-	-	-	+	-	+
Yam	-	-	-	-	-	+	+
Eba	-	-	-	-	+	+	+
Kati	-	+	+	-	-	-	+
Fufu	-	+	-	-	-	-	+
Eko	-	-	-	+	-	-	+

'+' and '-' mean present and absent respectively. *Kleb.= Klebsiella aerogenes, Lact.= Lactobacillus plantarum, Leuc.= Leuconostoc* sp., *Micr.= Micrococcus varians, Prot.= Proteus mirabilis, Strep.= Streptococcus faecali, Staph.= Staphyloccus epidermidis.*

Table 2. Occurrence and distribution of fungi in the food samples.

Food sample	*A. fla*	*A. nig*	*Clad*	*Geot*	*Muco*	*Neur*	*Peni*
Pounded yam	-	-	-	-	-	+	+
White rice	-	-	-	+	-	+	-
Beans	+	-	-	+	-	-	-
Yam	-	+	+	-	-	-	-
Eba	-	+	-	-	+	-	+
Kati	-	-	-	-	-	-	-
Fufu	+	-	-	-	-	-	-
Eko	+	-	-	+	-	+	+

'+' and '-' mean present and absent, respectively. *A. fla= Aspergillus flavus, A. nig= Aspergillus niger, Clad= Cladosporium herbarum, Geot= Geotrichum candidum, Muco= Mucor mucedo, Neur= Neurospora sitophilia, Peni= Penicillium* sp.

had a load (5×10^4 CFU/mL) far lower than the pounded yam stored for 24 h (2.2×10^5 CFU/mL).

As shown in Figure 2, fungi colonization in the unfermented and fermented food samples was observed to commence within 48 and 72 h, respectively. At the end of 96 h storage, the fungal counts were observed to range from 1.8×10^3 propagules/ cells and 2.9×10^3 propagules /cells in the unfermented food samples. In the fermented food samples, fungal count was observed to range from 0 to 5.70×10^3 propagules/cells, respectively. In all the fermented foods, there were no traces of fungal growth for 48 h. Throughout the 96 h storage period, no fungi colonization was observed in 'kati' (Figure 2). As was observed with the bacterial counts, the fungal counts in the unfermented food samples were significantly higher than counts in the fermented food samples ($P \leq 0.05$).

During the storage period, a total of eight bacterial strains were isolated from the food samples. The isolated bacterial strains include *Klebsiella aerogenes*, *Lactobacillus plantarum*, *Leuconostoc* sp., *Micrococcus varians*, *Proteus mirabilis*, *Streptococcus faecalis* and *Staphylococcus epidermidis* (Table 1). In the case of fungi, eight strains were isolated from the food samples. The strains were *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium herbarum*, *Geotrichum candidum*, *Mucor mucedo*, *Neurospora sitophilia* and *Penicillium* sp. (Table 2).

With respect to the frequency of occurrence of microbial isolates from the food samples, a rank of the bacteria revealed that *Leuconostoc* sp (13%) and *Staphylococcus epidermidis* (45%) were the least and the most occurring bacteria in the food samples, respectively. In the case of fungi, *Aspergillus niger* and *Penicillium* sp. were observed to be the least (40%) and the most occurring fungi in the food samples, respectively (Figure 3).

With respect to the organoleptic attributes of the food samples during storage, although there was a significant difference between the different food samples, a general trend was that the fermented food samples were more acceptable to the panellists than the unfermented food samples. Only a few number of respondents accepted

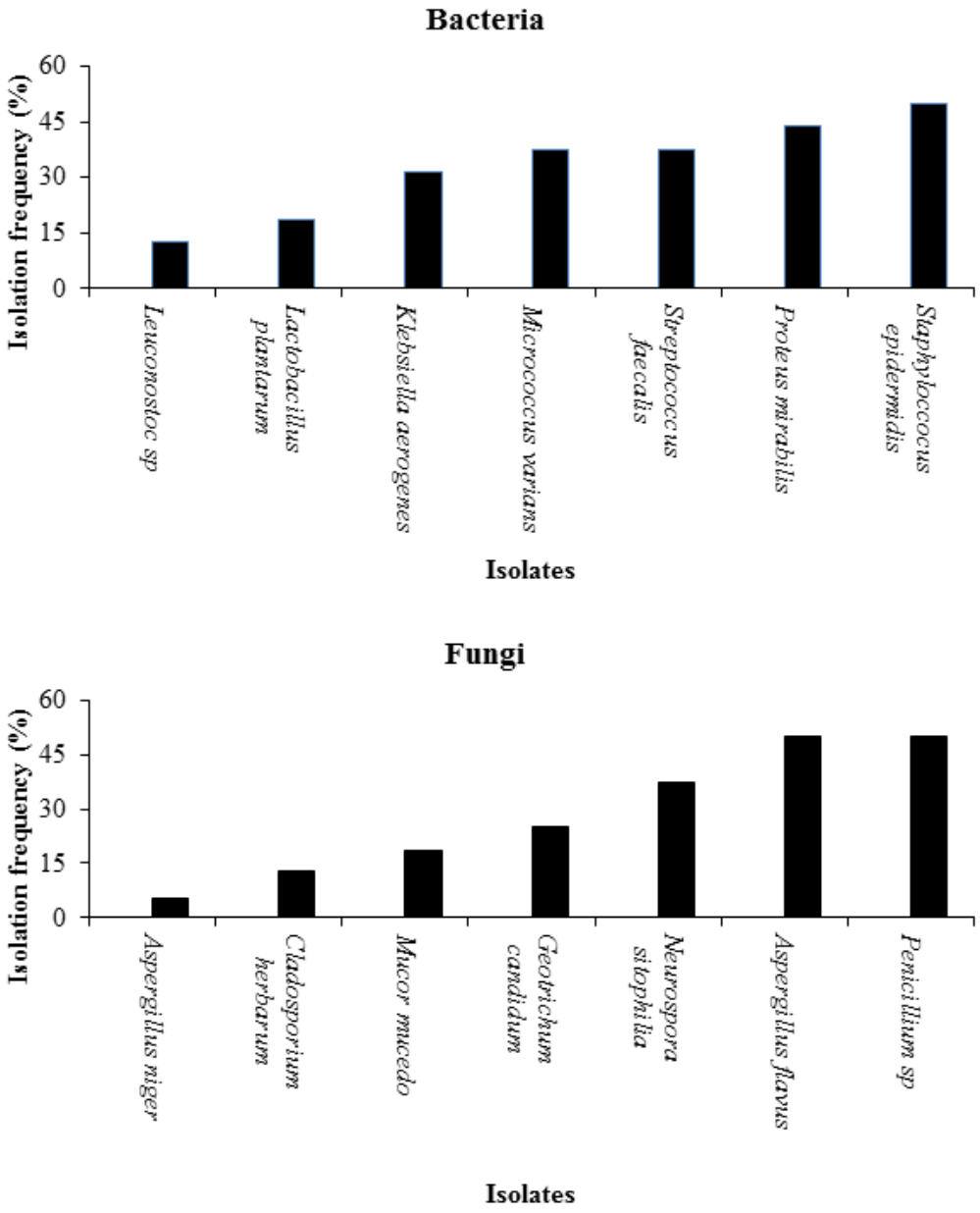

Figure 3. Rank of the frequency of occurrence of the microbial isolates in the food samples.

beans stored for 96 h while 'Kati' was the most acceptable of the food samples. A daily decrease in the means of the attribute was observed in all the unfermented food samples while the arithmetic difference in the means of the fermented food samples was observed to be negligible (Table 3).

A comparison of storage at 25 and 4°C revealed that off-colour, mouldiness and objectionable taste were the commonest indicators of spoilage in all the food samples. Stringiness and sliminess were observed to be limited to the unfermented food samples. The results revealed that all the food samples were affected by one or more fungal species as shown by mouldiness (Table 4). The foods stored at refrigeration temperature had longer acceptable days (5 to 76 days) and a longer shelf life (4 to 56 days) than those stored at room temperature, which were 2 to 22 days and 2 to 17 days for acceptable days and longer shelf life, respectively).

DISCUSSION

A general observation in this study was the presence of higher microbial load during storage in the unfermented food samples than in the fermented. The higher storability and lower spoilage rate of the fermented food samples are therefore not surprising as fermented foods had been

Table 3. General Sensory evaluation of the stored food samples.

Food sample	Fresh	24 h	48 h	72 h	96 h
Pounded yam	9.8[a]	5.6[c]	4.7[c]	2.6[d]	1.5[d]
Yam	9.6[a]	6.0[c]	5.4[b]	4.6[b]	2.4[c]
Rice	10.0[a]	6.5[b]	5.1[c]	3.5[c]	1.98[c]
Beans	9.6[a]	6.1[c]	4.8[c]	2.5[d]	1.2[d]
Eba	9.9[a]	8.6[a]	8.5[a]	8.0[a]	7.0[b]
Kati	9.9[a]	8.5[a]	8.5[a]	8.2[a]	8.0[a]
Fufu	9.8[a]	8.3[a]	8.0[a]	7.7[a]	7.5[b]
Eko	10.0[a]	8.2[a]	8.2[a]	7.8[a]	7.5[a]

Values in the same column not followed by the same superscript are significantly different ($p \leq 0.05$).

Table 4. Observed days of spoilage symptoms of the food samples stored at 25 and 4°C.

Parameter	Pounded yam	Yam	Rice	Beans	*'Eba'*	*'Kati'*	*'Fufu'*	*'Eko'*
Appearance								
Off-colour	3 (4)	2 (3)	2 (4)	4 (6)	5 (17)	17 (56)	9 (35)	5 (15)
Mouldiness	4 (7)	3 (6)	5 (8)	5 (9)	7 (20)	20 (64)	12 50)	8 (21)
Texture								
Stringiness	3 (-)	2 (-)	2 (5)	- (-)	- (-)	- (-)	- (-)	- (-)
Sliminess	- (-)	1 (4)	2 (4)	1 (4)	- (-)	- (-)	- (-)	5 (-)
Watery	3 (-)	- (-)	2 (5)	3 (-)	- (-)	- (-)	- (-)	7 (-)
Softness	2 (-)	3 (-)	2 (4)	- (-)	6 (20)	- (-)	9 (-)	6 (-)
Separated	2 (3)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	7 (18)
Aroma								
Off-odour	2 (4)	2 (4)	2 (4)	2 (5)	7(22)	19 (74)	12(50)	8 (17)
Putrid	- (-)	- (-)	2 (3)	2 (5)	- (-)	- (-)	- (-)	- (-)
Taste								
Objectionable	3 (4)	2 (4)	1 (5)	2 (6)	5 (21)	22 (72)	10(62)	9 (19)
Sourness	2 (3)	- (-)	- (-)	- (-)	- (-)	18 (70)	- (-)	9 (5)
Rejected	2 (5)	2 (5)	3 (7)	2 (7)	8 (22)	22 (76)	15(52)	11(24)

Values in parenthesis represent storage at 4°C while all other values indicate at 25°C. '-' indicate not detected. *'Kati' and 'Eko'* were wrapped in leaves during the storage period.

reported to have a higher shelf life (Steinkraus, 1997; Ogunbanwo et al., 2004). This could be attributed to the release of antimicrobial metabolites by the fermenting organisms into these food substrates (Adebayo and Aderiye, 2010). The antimicrobial compounds include bacteriocin, diacetyl, hydrogen-peroxide and organic acid (Adebayo and Aderiye, 2007).

The differences in the microbial contamination of pounded yam and yam both from the same raw material could be attributed to the higher level of moisture content in the pounded yam. Frazier and Westhoff (2007) had reported that high water activity favoured the growth of contaminating microorganisms. The higher storability of the refrigerated food samples (4°C) could be attributed to the fact that the spoilage organisms are mesophiles hence the low temperature (4°C) inhibited their metabolic activities and thus led to limited growth, inhibition or death in causes of some organisms (Bracket, 1997). Also, the highest shelf life (76 days) recorded by 'Kati' may be due to its low pH, low moisture and high fibre contents. Such food tends to be more stable microbiologically (Frazier and Westhoff, 2007). One of the findings of this study, that mouldiness is a perceived indicator of deterioration in the selected Nigerian foods, predicates

the need to evolve means to prevent fungal spoilage of fermented Nigerian foods.

A total of seven fungal strains belonging to six genera were found to be responsible for the spoilage of the food samples used in this study. The most predominant genera were *Aspergillus* and *Penicillium* which constituted more than 66% of the total fungal isolates. Previous workers have reported the predominance of these organisms in other food systems like mawe (Hounhouighan et al., 1993); 'burukutu' (Sanni et al., 1999) and dried sausages (Mataragas et al., 2002).

The prevalence of *Aspergillus* and *Penicillium* species could be due to their sporulating ability; hence they easily contaminate the environment (Frisvad and Samson, 2007). Food samples from 'Eko' recorded the highest number of fungal contamination (66.66%). This could be attributed to its having the highest moisture content (55.04%). Foods with high moisture content generally encourage fungal growth because spore germination and hyphal growth require moisture (Odigie, 2000). Another reason could be due to the wrapping of 'Eko' with leaves of *Thaumatococcus daniella*, which could be a potential source of contamination. According to Fadahunsi and coworkers (2013), the occurrence of the isolates could be attributed to the biodegrading potential of the microflora to convert diverse substrates by secretion of extracellular enzymes.

In the present study, the spoilage fungi isolated from the food samples are common microorganisms associated with the environment. Contamination of the food substrates by these fungi was expected as the ambient temperature of storage and pH (4.1-4.6) of the fermented food samples favour fungal proliferation (Brock and Madigan, 2003). Many of the isolated fungi are of clinical importance because of their potentials in causing human diseases. For instance, strains of *Aspergillus* and *Penicillium* spp. are known to produce mycotoxins which can result in mycotoxicoses (Smith et al. 1995). The toxins can damage liver, causing cirrhosis and can also induce tumor. Hence the assumption of some consumer of the fermented foods in southwest Nigeria that these mould - infested foods are only unaesthetic but not dangerous to health should be discouraged, as such foods could be potential sources of mycotoxicosis.

The 'Eko' had the highest occurrence and distribution of genera of fungal contaminants (5) while 'Fufu' had the least (2). The differences in the occurrence of these fungi in the food samples may be attributed to the differences in their nutrient status. For instance, Adebayo and Aderiye (2009) reported that 'Eko' had higher metabolisable sugar (1.63%) and moisture (55.04%) content, than 'Fufu' (0.048 and 42.15%) thus predisposing the former to a higher level of fungal attack. The delay in the incidence of the spoilage organisms until after 72 h could be attributed to inadequate moisture level required for spore germination prior to 72 h of storage or to the antimicrobial effect of some compounds liberated by LAB into the food substrates during fermentation (Ryhz et al., 2008; Adebayo and Aderiye, 2010).

Foods generally become more susceptible to pathogens as storage progressed due to the absorption of moisture from air and thus make the tissues more accessible to hydrolytic enzymes of the attacking pathogens. It is indicated that although microbial fermentation has played a vital role in food processing in Nigeria, because many of the indigenous fermented foods are processed through spontaneous and uncontrolled natural fermentation, despite its advantages, the accompanying microflora are indicated to be causes of spoilage and in some case toxicity (Aderiye and Adebayo, 1999).

Infestation by spoilage fungi caused some physical defects like off-colour, loss of firmness, loss of aroma, and softness on the food samples. Frazier and Westhoff (2007) attributed the change in texture to the activity of the spoilage organisms that encouraged the extraction of mineral nutrient from the food substrates. Loss of aroma was however attributed to the fermentation of the non-proteinaceous constituents of the food substrates. Fungal activity has been reported to cause a rapid deterioration of quality of food substrates (Teniola and Odunfa, 2002; Samson, 2007; Adebayo and Aderiye, 2009).

Conflict of Interests

The author(s) have not declared any conflict of interests.

Conclusion

This study which was aimed at evaluating the microbial spoilage of selected fermented and unfermented foods in Nigeria have revealed that during storage, both fermented and unfermented foods are susceptible to spoilage, although the onset of spoilage is faster in the unfermented one. A variety of bacteria and fungi species were also observed to be responsible for spoilage of the food samples during storage. With storage at room temperature, the onset of spoilage was remarkably faster in the fermented food samples than the fermented ones. In all, a sensory evaluation showed that the fermented foods were more acceptable to panelists than the unfermented ones after storage. This could indicate that the palatability of the fermented food samples only experienced slight or no changes during storage, when compared to the unfermented ones.

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