

## 1 Original Research Article

# 2 The predominant lactic acid microorganisms of 3 spontaneously fermented *amala*, a yam food 4 product

## 5 ABSTRACT

6 **Aim:** The present study is focused on determining if there are differences in the types of  
7 organisms responsible for spontaneous fermentation in four (4) varieties of yam (*Dioscorea*  
8 *rotundata*), namely, TDr Pepa, TDr Amila, TDr Alumaco and TDr 95/19177, while also  
9 ensuring that the expected organoleptic properties associated with the fermentation process  
10 from this study location is reproducible.

11 **Study Design:** A Complete Randomized Design (CRD) with three replications was adopted  
12 and used to test for significant differences between the two cassava products.

13 **Place and Duration of Study:** The tubers of yam were obtained from the International  
14 Institute for Tropical Agriculture (IITA), Ibadan, and were processed at Ede, Nigeria between  
15 March and May 2016.

16 **Methodology:** Using standardized spontaneous fermentation methods, the two varieties of  
17 yam, were sampled eight hourly over a period of 24 hours, for lactic acid bacteria and fungi.  
18 Samples were incubated anaerobically, representative microbial populations were enumerated  
19 and identified using standard microbiological protocols. Sensory evaluations were conducted.

20 **Results:** The results showed that the only isolated predominant lactic acid bacterial organism  
21 was *Lactobacillus brevis*. On the other hand, the representative lactic acid fungal isolates  
22 were identified as *Rhizopus spp*, *Aspergillus niger*, *Aspergillus flavus* and *Neurospora spp*.  
23 Investigation of succession organisms revealed slight differences between the sun-dried and  
24 oven-dried *amala* samples, although the differences were not statistically significant at  $p \leq$   
25 0.05 using a one-way analysis of variance ANOVA.

26 **Conclusion:** The present results show that in spite of the spontaneity of the fermentation  
27 process, the different yam varieties supported the growth and reproduction of similar  
28 fermentation organisms. Furthermore, the prevailing microenvironment in the fermentation  
29 set up appears to be the most important factor in determining the predominating organisms in  
30 the fermentation process and the organoleptic characteristics of the final product. Results  
31 from this study show that it is possible to reproduce the organoleptic characteristics peculiar  
32 to this test location using the isolated lactic acid microorganisms.

34 **Key Words:** Lactic acid bacteria, *Dioscorea rotundata*, Food security

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## 37 INTRODUCTION

38 Yam (*Dioscorea spp*) occur in Asia, East Africa, the Caribbean, India, Tonga, south  
39 pacific as well as West Africa [1]. It is estimated that yam consumption yearly is over  
40 48million tones globally, out of which Nigeria alone produces 67-70% of global yam supply,  
41 followed by Ghana, Ivory Coast and Togo [2, 3]. Both fresh tubers and yam flour are now  
42 exported from Ghana and Nigeria to developed countries such as United Kingdom and  
43 United States of America where the patrons are mainly emigrants from the yam growing  
44 regions.

45 Yam is considered to be a food security crop particularly in West Africa where it is  
46 estimated to provide more than 200 dietary calories each day for over 60 million people [4].  
47 Food security is a condition that exists when individuals at all times have economic and  
48 physical access to safe, nutritious and sufficient food in order to meet their dietary needs and  
49 food preferences for an active and healthy life [5]. As a food security crop in Africa, yam is  
50 third in line after cassava and maize where the demand for this commodity increases as  
51 incomes increase as consumers shift from other carbohydrate substitutes to yam, especially  
52 when the price of yam relative to price of its substitutes declines [2].

53 However, much of the tuber yield is lost to postharvest diseases caused by bacteria  
54 and fungi under the poor storage conditions that exist in the yam producing areas. For  
55 example, losses caused by pathogens attack vary from 20-30% generally in some crops [6].  
56 Moreover, in the absence of good storage facilities which is a prevalent condition in yam  
57 growing regions, yam tubers are prone to gradual physiological deterioration after harvesting.  
58 These physiological and biochemical changes are known to occur which often reduce the  
59 food quality of tubers [7]. On the other hand, yams can be processed into less perishable  
60 products such as yam flour through a drying process. The flour can later be reconstituted with

61 hot water to form paste or dough. The reconstituted flour known as *Amala* is popular for  
62 feeding both adults and children, and it is an important source of carbohydrate for many  
63 people in the yam zone of West Africa [8]. Yam flour can be easily stored for a long period  
64 (12 - 18 months) if the flour is free from moisture; hence yam is commonly processed into  
65 flour by drying yam slices and milling.

66         In spite of the elite status of yam as a staple food and its renowned ability to provide  
67 the appropriate calories, it is poor in other nutrients. For example, Protein calorie malnutrition  
68 (PCM) is widely prevalent in Africa, particularly among rural women and children that  
69 subsist mostly on yam and other carbohydrate food sources such as cassava and maize [9].  
70 Fermentation of yams to produce flour has been found to improve product nutritional quality  
71 and organoleptic properties of the final product [10]. The processing of yams traditionally  
72 depend on the species, for example, *Dioscorea alata* is always preferred for use in preparing  
73 porridge, whereas *Dioscorea rotundata* is always preferred for use in preparing boiled yam,  
74 pounded yam and yam flour [8]. Varieties of *Dioscorea rotundata* were processed into *elubo*  
75 (yam flour) and further made into *amala* (the ready-to-eat paste made from *elubo*) in the  
76 present study.

77         Yam flour processing is similar among the West African countries, such as Nigeria,  
78 Benin and Ghana. This involves peeling dry yam tubers, sometimes slicing, parboiling in hot  
79 water (at 40–60 °C for 1–3 h), steeping for about a day and sun drying. The parboiled, steeped  
80 and sun-dried product is called “*gbodo*” in the Yoruba land of Nigeria. When milled into  
81 flour, “*gbodo*” is called “*elubo*”, which when stirred into boiling water to make a thick paste  
82 is known as “*amala*” [11]. “*Amala*” is usually eaten with soup by consumers [12, 13]. The  
83 main quality attributes of *amala* are colour, texture and taste [13]. Most consumers prefer a  
84 light brownish, elastic, nonsticky *amala* with a slightly sweet taste, while a slightly bitter  
85 taste is also tolerated [13, 14, 15].

Traditionally, sun-drying in the open is the most popular method employed in yam flour production as it represents a low cost processing method of preserving agricultural produce in the tropics. This drying method however has some limitations. These include the inability to control the drying process and parameters, weather uncertainties, high labour costs, the requirement of a large drying area, insect infestation, and contamination with dust and other undesirable materials [16]. A controlled environment, is therefore, recommended to improve the quality of the product. Hot air drying in a controlled environment is a method in which heated air is blown over food materials with the aid of fan(s) to remove most of the moisture from the food material. The drying of wet materials induces a number of physico-chemical changes in the product, often reflected by colour.

Lactic acid fermentation is commonly used in many parts of the world as a method for preservation of plant materials as well as obtaining desirable sensory and nutritional properties to the product [17]. The present is focused on standardizing the *elubo* making process, particularly in order to ensure consistency in nutritional quality, taste and other organoleptic properties of the final product made in the geographical location of the present study since the fermentation is spontaneous. Consequently, the microflora and the effect of spontaneous lactic acid fermentation and the causal organisms on the proximate, nutritional, sensory and visual characteristics of the spontaneously fermented yam were investigated.

## **MATERIALS AND METHODS**

### *Collection of samples*

Four different varieties of yam samples, namely, TDr Pepa, TDr Amila, TDr Alumaco and TDr 95/19177 were obtained from the International Institute of Tropical Agriculture at Oyo Road, Ibadan, Oyo state, Nigeria.

109           The yam samples were washed, peeled, diced, soaked in water at 50°C for 24 hours,  
110       dried, milled to flour and then sieved, this was done according to the method described by  
111       Babajide *et al.* [18]. The flow chart used in processing the yam tubers into yam flour is  
112       shown in Chart 1. Microbial and proximate samples were taken for sample analyses within 24  
113       hours of steeping.

#### 114       *Identification of isolates*

115           Microbiological analyses were conducted immediately after sampling. Sampling was  
116       done by agitating the steeped yam before sampling to ensure uniform mixing, then, to 1mL of  
117       the sampled solution 90 ml of sterile normal saline was added, vortexed and further diluted in  
118       a 10-fold dilution series. For Lactic acid bacteria, 0.1 ml of suitable dilutions of inocula were  
119       spread onto De Man Rogosa Sharpe (MRS) agar, plates were incubated anaerobically at 30°C  
120       for 24 h in an anaerobic incubator (Surgical Medical England Model SM-80CH, uv).  
121       Representative dominant colonies were picked from the plates of the suitable dilutions and  
122       purified by repeated streaking onto nutrient agar. For lactic acid fungi, 0.1 ml of suitable  
123       dilutions of inocula were spread onto Potato Dextrose agar (PDA). Eight hourly changes over  
124       a period of 24 h in the microbial population of the total viable lactic acid bacteria and fungi  
125       were determined using MRS agar and PDA respectively. Samples were enumerated by using  
126       appropriate sterile dilution and spread plate methods eight hourly. For the identification of  
127       microbial isolates, the fungal plates were incubated at 25°C for 2-5 days, while the bacteria  
128       were incubated at 30°C for 24- 48 h. Three colonies for each morphological type was purified  
129       and maintained in the appropriate agar plates. Systematic morphological and biochemical  
130       tests were conducted according to [19, 20], moreover, identification of bacterial isolates into  
131       species was done according to tests and descriptions given in [21] and [22]. The fungal  
132       isolates were characterized by their cultural properties stained with cottonblue lactophenol  
133       solution and observed microscopically [23].

### 134 *Organoleptic analysis*

135 For the sensory evaluation (colour, aroma and texture), the *amala* obtained on zero  
136 fermentation was poured into container labelled 0 h, *amala* of 8 h of fermentation into  
137 container labelled 8<sup>th</sup> hour, *amala* of the 16<sup>th</sup> hour of fermentation into the container labelled  
138 16<sup>th</sup> hour, and so on till the 24<sup>th</sup> hour. A panel of thirty individuals were invited for the  
139 sensory evaluation (organoleptic appeal) of odour, taste, appearance, pasting, texture and  
140 general acceptability. The samples in the container were presented to the evaluators at  
141 random. The evaluators were asked to award scores for each sample after observing the  
142 colour, aroma and texture of each sample. The products were ranked on a scale of 1-5; 1 –  
143 extremely dislike, 2- dislike, 3- neither like nor dislike, 4- like and 5- like extremely.

### 144 *Oven drying versus sun drying*

145 Since colour is one of the quality parameters investigated in the present study, the  
146 *elubo* samples tested were dried under the two drying regimes of sun and oven drying in  
147 order to determine the effects of the drying method used on the final product. After steeping  
148 the yam slices for 0, 8, 16 and 24 hours (see Chart 1), the blanched slices were divided into  
149 two sets, one set was sun-dried for two weeks and the other set was oven-dried to constant  
150 weight in a convective air dryer operated at 60°C at an air velocity of 2.5 ms<sup>-1</sup> until constant  
151 weight was obtained. The dried slices were milled with a hammer mill and then sieved using  
152 a laboratory sieve of 600 mm aperture size.

### 153 *Experimental Design*

154 Complete Randomized Design (CRD) with three replications was used to test if  
155 spontaneous fermentation of yam improves the organoleptic characteristics of *amala* made  
156 from sun or oven-dried yam. These characteristics include odour, taste, appearance, pasting,  
157 texture and general acceptability. The results of the three replicates were pooled and  
158 expressed as mean  $\pm$  standard error (S. E.). A one-way analysis of variance (ANOVA) and

the least significance difference (LSD) were carried out. Significance was accepted at  $p \leq 0.05$  using SPSS software version 21.0.

## RESULTS

### *Isolation and identification of lactic acid bacteria and fungi from the fermented products*

Table 1 shows the identification table of representative lactic acid bacterial isolates from the anaerobic culturing of samples from the fermentation of yam for the production of *elubo* in the two varieties of yam, namely, TDr Alumaco and TDr Pepa. The other two varieties of yam used in this study, namely, TDr Amila and TDr 95/19177 exhibited no growth of lactic acid bacterial from the anaerobic culture of samples during the fermentation process. The representative isolates were grouped based on cultural characteristics, gram staining and biochemical test results. The same predominant lactic acid bacterial organism was found in the two varieties of yam (TDr Alumaco and TDr Pepa). The organism was identified as *Lactobacillus brevis* (Table 1).

The identification table of representative lactic acid fungal isolates from the anaerobic culturing of samples from the fermentation of yam for *elubo* for the four varieties of yam, namely, TDr Alumaco, TDr Pepa, TDr Amila and TDr 95/19177 is shown in Table 2. The representative isolates were grouped based on cultural, morphological characteristics and results of standard biochemical reaction. The results showed that irrespective of the variety of yam, the predominant organisms remained the same. The four (4) organisms were identified as *Rhizopus spp*, *Aspergillus niger*, *Aspergillus flavus* and *Neurospora spp*. Moreover, the results show that the fungal organisms occur in the following order from the most highly occurring to the least occurring: *Aspergillus niger*, *Neurospora spp*, *Aspergillus flavus* and *Rhizopus spp* (Table 3). In addition, the yam varieties with the highest load of lactic acid fungi were TDr Alumaco and TDr Amila (Table 3).

### *Succession of organisms*

Table 3 shows the percentage frequency of isolation of the organisms encountered during the spontaneous fermentation process for *elubo*, the fungal organisms identified as *Rhizopus spp*, *Aspergillus niger*, *Aspergillus flavus* and *Neurospora spp*. were the predominant starter organisms isolated from the *elubo* samples with incidence values ranging from  $0.1 \times 10^{-11} - 3.4 \times 10^{-8}$ cfu/ml of samples. These ranges were consistently obtained for samples obtained from all the varieties of yam used in the present study. On the other hand, the only lactic acid bacteria isolated in this study was identified as *Lactobacillus brevis*. *L brevis* was found to occur in the yam varieties TDr Alumaco and TDr Pepa where this particular lactic acid bacterium was too numerous to count within the first eight hours of the fermentation process but subsequently declining to zero growth by the 16<sup>th</sup> hour.

Moreover, Fig. 1 shows that a gradual reduction in the number fungal isolates as the fermentation progressed in all the yam varieties. The highest incidence values were observed in the TDr95/19177 variety, followed by TDr Alumaco, TDr Amila and TDr Pepa in descending order.

Table 4 shows the results of the organoleptic tests on *amala* samples processed from the four varieties of yam that were either processed by sun or oven drying, the results showed that for odour, the *amala* made from sun-dried yam variety TDr 95/ 19177 was the most preferred while *amala* from sun-dried TDr Alumaco was most preferred for general acceptability. The indicated values are average scores of triplicates, n=30. However, there were no significant differences when the recorded values were compared statistically at  $p \leq 0.05$  using a one-way analysis of variance ANOVA.

## DISCUSSION

The identities of the fermentation organisms isolated from the present study confirm similar studies that were done on yam fermentation, notably, the works of [24, 25] identified similar organisms from spontaneous fermentation of yam. Moreover, the present result goes further

to show that there may be differences in organoleptic appeal due to the type of drying method employed in processing the yam flour before being made into *amala*. The results showed a slight preference for the sun-dried yam, although these differences were not found to be statistically significant. In addition, the results on Table 4 showing that TDr Alumaco as the most appealing in terms of general acceptability, followed by TDr Amila, TDr 95/19177 and TDr Pepa, in descending order of general acceptability will be of value in scale up experiments to determine which variety of yam would be most promising for use in industrial (large scale) production of yam flour meal, *amala*.

The succession data presented in this study (Figs 1 and Table 3) shows that the lactic acid bacteria, *Lactobacillus brevis* and lactic acid fungi, *Rhizopus spp*, *Aspergillus niger*, *Aspergillus flavus* and *Neurospora spp*. are promising candidates for subsequent pilot studies in order to optimize the organoleptic appeal of *amala*. This is well corroborated by previous reports where various species of *Lactobacillus* including *L. brevis*, *L. plantarum*, *L. delbruecki* etc. were found to predominate yam fermentation in *amala* processing [24, 26, 27]. In fact it was concluded that the success of *L. plantarum* to predominate in cassava fermentation demonstrates the potential for its development as starter cultures for yam flour (*elubo*) industrialization. It is notable that success has been achieved in the use of lyophilized LAB strains as starter cultures for another indigenous African fermented food from cassava, namely, *gari* production has been reported, where *L. plantarum* produced at low cost has been reportedly used in large-scale production of *gari* [28].

Moreover, the identification of lactic acid fungi such as *Rhizopus spp*, *Aspergillus niger*, *Aspergillus flavus* and *Neurospora spp* is well corroborated by earlier report by Babajide et al, 2015 where different species of *Aspergillus* and *Rhizopus* were identified from steeped yam fermentation [25].

## CONCLUSION

The present results show that in spite of the spontaneity of the fermentation process, there are similarities in the type and amount of isolated lactic acid microorganisms from all the varieties of yam evaluated in the present study. This result confirms that lactic acid bacteria and fungi have the ability to adapt to many different substrates. In addition, the results show that the prevailing microenvironment in the fermentation set up is the most important factor in determining the predominating organisms in the fermentation process and the organoleptic and nutritional characteristics of the final product. Moreover, the present results demonstrate the successful isolation of the lactic acid bacteria, *Lactobacillus brevis* and lactic acid fungi, *Rhizopus spp*, *Aspergillus niger*, *Aspergillus flavus* and *Neurospora spp* as promising candidates for subsequent pilot studies in order to optimize the organoleptic and nutritional characteristics of *amala*. These results indicate that it is possible to reproduce the organoleptic and nutritional characteristics peculiar to this test location with the aid of the identified lactic acid microorganisms.

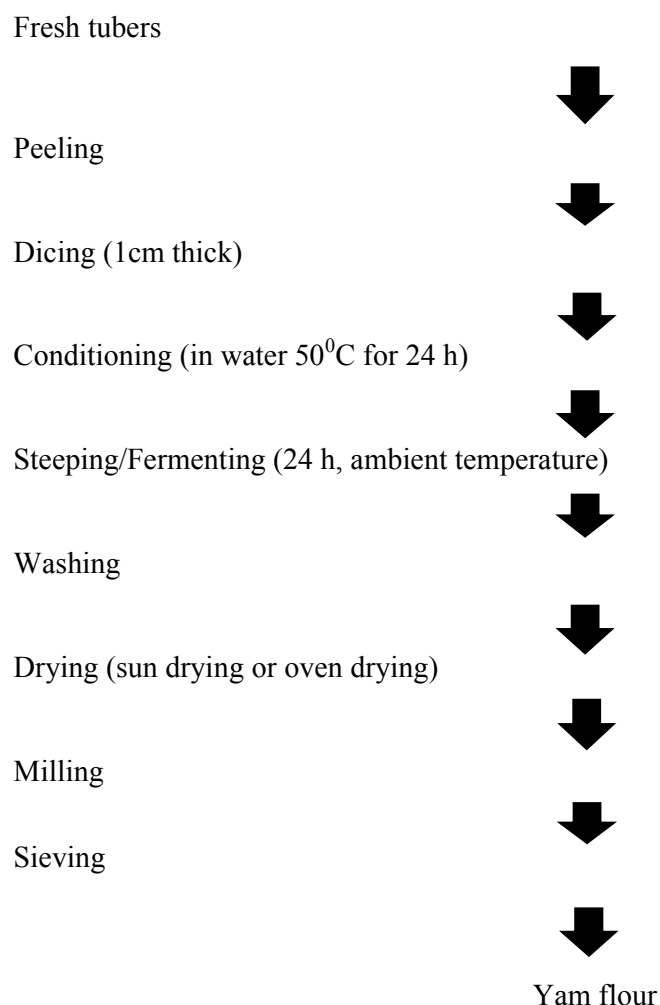
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**Chart 1: The flow chart for the production of yam flour.**



**Table 1: The morphological and biochemical characteristics of the identified lactic acid bacterial isolates from the spontaneous fermentation of two yam varieties**

Yam variety	Gram staining	Morphology	Catalase	Methyl Red	Arginine Decarboxylase	Mannitol	Maltose	Sucrose	Lactose	Glucose	Fructose	Arabinose	Ribose	Identified organism
TDr Alumaco	+	Rod	+	+	+	- No gas	+	+	-	+	+	+	+	<i>Lactobacillus brevis</i>
TDr Pepa	+	Rod	+	+	+	- No gas	+	+	-	+	+	+	+	<i>Lactobacillus brevis</i>

**Table 2: Identification table for the lactic acid fungal isolates from the spontaneous fermentation of four yam varieties, namely, TDr Alumaco, TDr Pepa, TDr-95/19177 and TDr Amila**

Organism	Morphological Characteristics	Microscopic Morphological Characteristics	Identified Organism
1	Large fluffy white milky colonies which later turns black as culture ages.	Non-septate hyphal with uptight sporangiophore connected by stolon and Rhizopus, dark pear shaped sporangium on hemispherical columella.	<i>Rhizopus spp</i>
2	Black spores with cream mycelia edges, same on reverse plate.	Hyphae is septate. Spore bearing.	<i>Aspergillus flavus</i>
3	Colonies of felt like yellow to white hyphae, turning black with the formation of conidia.	Hyphae is septate, hyaline acute-angle branching. Conidial head biserial, radiate, conidia in chains or detached and dispersed.	<i>Aspergillus niger</i>
4	Cream yeast-like	Hyphae is non-	<i>Neurospora spp</i>

spores, same on  
reverse plate.

septate.  
Conidiophores are  
branched and  
smooth. Head is  
radiated.

350

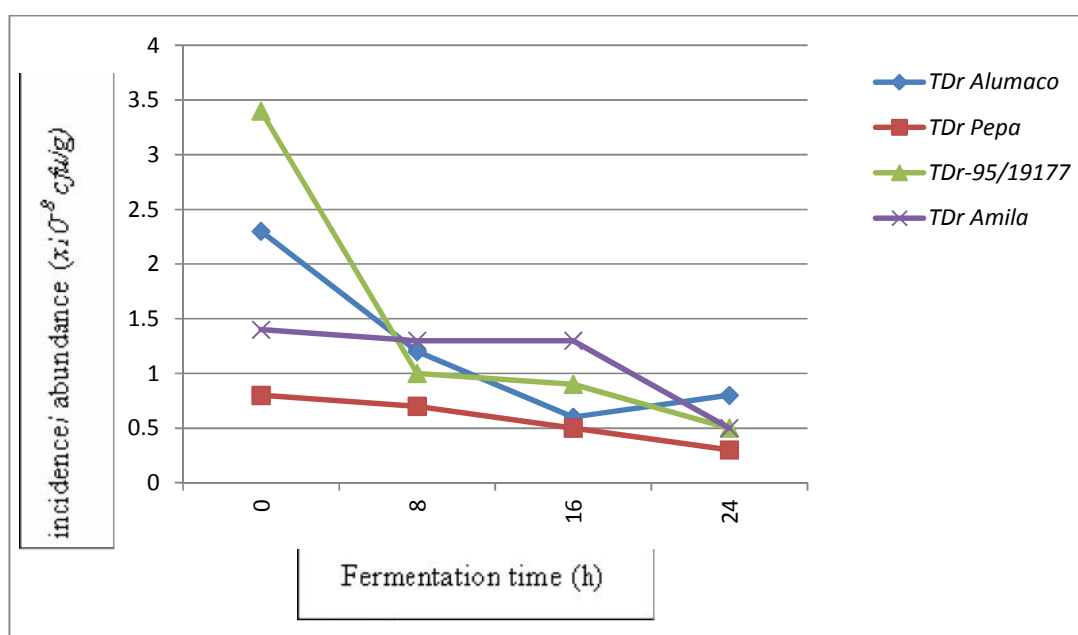
351 Table 3: The occurrence (%) of the fungal isolates in the four yam varieties sampled during  
352 spontaneous fermentation

Yam variety	Alumaco				Pepa				Amila				TDr95/19177				Total isolates
Fungal Species	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
<i>Rhizopus spp</i>	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	3
<i>Aspergillus flavus</i>	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	12
<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	14
<i>Neurospora spp</i>	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	13
Total organisms	2	3	3	3	3	3	3	2	3	3	3	1	3	4	2	1	42
Total Fungal count (cfu/g)	2.3 $\times 10^{-11}$	1.2 $\times 10^{-11}$	0.6 $\times 10^{-11}$	0.8 $\times 10^{-11}$	0.8 $\times 10^{-8}$	0.7 $\times 10^{-8}$	0.5 $\times 10^{-8}$	0.3 $\times 10^{-8}$	3.4 $\times 10^{-8}$	1.0 $\times 10^{-8}$	0.9 $\times 10^{-8}$	0.1 $\times 10^{-8}$	1.4 $\times 10^{-11}$	1.3 $\times 10^{-11}$	1.3 $\times 10^{-11}$	0.1 $\times 10^{-11}$	

353

354 Legend: A= 0hr after fermentation; B= 8hr; C= 16hr; D= 24hr

355 Fig 1: A line graph tracking the typical incidence/ abundance ( $\times 10^{-8}$  cfu/g) of the lactic acid  
356 fungi from four varieties of yam (TDr Alumaco, TDr Pepa, TDr-95/19177 and TDr Amila)  
357 during *elubo* fermentation.



358

Table 4: Organoleptic appeal test results of *amala* prepared from flour processed from sun-dried and oven-dried yam (in parenthesis)

Sample source	Odour	Taste	Appearance	Pasting	Texture	General Acceptability
TDr Pepa	3.7 (3.2)	4.0 (4.1)	4.1 (4.1)	4.2 (3.9)	4.3 (4.1)	<b>4.0</b> <b>(4.1)</b>
TDr Amila	3.8 (3.5)	3.9 (4.0)	3.8 (3.5)	4.1 (4.3)	4.1 (4.0)	<b>4.3</b> <b>(4.2)</b>
TDr Alumaco	3.8 (3.6)	4.1 (4.2)	4.3 (4.1)	4.5 (4.3)	4.4 (4.1)	<b>4.5</b> <b>(4.3)</b>
TDr 95/ 19177	4.1 (3.8)	4.3 (4.1)	4.1 (4.3)	4.4 (4.4)	4.1 (4.3)	<b>4.1</b> <b>(4.3)</b>

The indicated values are average scores of triplicates, n=30. However, there were no significant differences when the recorded values were compared statistically at  $p \leq 0.05$  using a one-way analysis of variance ANOVA.