Asian Food Science Journal

4(3): 1-10, 2018; Article no.AFSJ.44088

The Predominant Lactic Acid Microorganisms of Spontaneously Fermented *Amala*, a Yam Food Product

Femi Ayoade[1*], Paulina, O. Adeniji[2], Kellanny, S. Amole[1], Yeitarere, A. Amaremo[1], Titilayo, O. Apata[1], Scott, O. Fayemi[1], Nicholas, E. Oyejide[1], Uchenna D. Abazuh[1], Tolulope, A. Kayode[1], Gbenga G. Daramola[3] and Onikepe, Folarin[1]

[1]Department of Biological Sciences, College of Natural Sciences, Redeemer's University, P.M.B. 230, Ede, Osun State, Nigeria.
[2]Department of Transport and Tourism Studies, College of Management and Social Sciences, Redeemer's University, P.M.B. 230, Ede, Osun State, Nigeria.
[3]Department of Chemical Sciences, College of Natural Sciences, Redeemer's University, P.M.B. 230, Ede, Osun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between the authors. Author FA designed the study, performed laboratory and statistical analyses, wrote the protocol and wrote the first draft of the manuscript. Author POA procured the cassava varieties and performed the proximate analysis in the laboratory. Authors KSA, YAA, TOA, SOF, NEO, UDA, TAK and GGD performed sample collection and laboratory analyses of the study. Author OF contributed to the development of protocol and provided technical support. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2018/44088
Editor(s):
(1) Dr. Amjad Iqbal, Assistant Professor, Department of Agriculture, Abdul Wali Khan University Mardan, Pakistan.
Reviewers:
(1) Sherif Mohamed El-Kadi, Damietta University, Egypt.
(2) R. Prabha, Dairy Science College, Karnataka Veterinary, Animal and Fisheries Sciences University, India.
(3) Olokun Alexander Lanzema, Bingham University, Nigeria.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/26401

Original Research Article

Received 23 June 2018
Accepted 12 September 2018
Published 27 September 2018

ABSTRACT

Aim: Using Four (4) varieties of yam (*Dioscorea rotundata*), namely, TDr Pepa, TDr Amila, TDr Alumaco and TDr 95/19177 differences in the types of organisms responsible for spontaneous fermentation were evaluated. The organoleptic properties of the final food products were also subjected to testing, in order to determine if these properties were reproducible.

**Corresponding author: E-mail: ayoadef@run.edu.ng;*

Study Design: Using a Complete Randomized Design (CRD) with three replications, the four varieties of yam were tested for significant differences in the characteristics of interest among the final products.
Place and Duration of Study: The present study was conducted between March and May 2016 at Ede. The yam tubers were sourced from the International Institute for Tropical Agriculture (IITA), Ibadan.
Methodology: In a standardised spontaneous fermentation set-up, four varieties of yam, were sampled eight hourly over a period of 24 hours, for lactic acid microorganisms. Representative microbial populations that were incubated anaerobically were isolated, counted, identified and characterised using standard microbiological protocols. The final products were evaluated for their organoleptic properties.
Results: The only isolated predominant lactic acid bacterial organisms was *Lactobacillus brevis*, while, *Rhizopus spp, Aspergillus niger, Aspergillus flavus and Neurospora spp* were identified as the representative lactic acid fungal isolates. The results revealed slight differences between the final products (*amala* samples) that were earlier processed using sun or oven-drying, although the differences were not statistically significant at p= .05 using ANOVA (one-way analysis of variance).
Conclusion: The present results confirmed that the prevailing microenvironment is the prime determinant of the predominant organisms in the fermentation process and consequently in the sensory properties of the final product. The present spontaneous fermentation data indicate that similar lactic acid organisms were isolated from the different yam varieties in the fermentation set up. The foregoing shows that the organoleptic characteristics specific to this test location may be reproduced using the isolated lactic acid microorganisms, perhaps on an industrial scale.

Keywords: Lactic acid bacteria; Dioscorea rotundata; food security.

1. INTRODUCTION

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

Yam is considered to be a food security crop particularly in West Africa where it is estimated to meet the daily calorific needs for tens of millions of the teeming population [4]. Food security is a condition that exists when individuals at all times have economic and physical access to their preferred food types under safe and nutritious conditions, and in sufficient quantities and as such able to maintain good health [5]. As a food security crop in Africa, yam is third in line after cassava and maize where the demand for this commodity increases as incomes increase as consumers shift from other carbohydrate substitutes to yam, especially when the price of yam relative to price of its substitutes declines [2].

However, much of the tuber yield is lost to postharvest diseases caused by bacteria and fungi under the poor storage conditions that exist in the yam producing areas. For example, losses caused by pathogens attack vary from 20-30% generally in some crops [6]. Moreover, in the absence of good storage facilities which is a prevalent condition in yam growing regions; yam tubers progressively deteriorate after harvesting. The progressive deterioration which are characterised as being physiological and biochemical in nature are known to reduce the food quality of tubers [7]. On the other hand, using drying processes yams can be salvaged from losses from deterioration by processing into less perishable products such as yam flour. The flour can later be reconstituted into paste or dough using hot water. The reconstituted flour known as *Amala* is popular for feeding both adults and children, and it is a major carbohydrate source for many people among the yam growing populations of West Africa [8]. Yam flour can be easily stored for up to a period of 18 months if the flour is free from moisture and for this reason yam is commonly processed into flour by drying yam slices and milling in these communities [9].

Inspite of the elite status of yam as a staple food and its renowned ability to provide the appropriate calories, it is poor in other nutrients.

For example, Protein calorie malnutriton (PCM) is widely prevalent in Africa, particularly among rural women and children that subsist mostly on yam and other carbohydrate food sources such as cassava and maize [10]. Fermentation of yams to produce flour has been found to improve product nutritional quality and organoleptic properties of the final product [11]. The processing of yams traditionally depend on the species, for example, *Dioscorea alata* is always preferred for use in preparing porridge, whereas *Dioscorea rotundata* is always preferred for use in preparing boiled yam, pounded yam and yam flour [8]. Varieties of *Dioscorea rotundata* were processed into *elubo* (yam flour) and further made into *amala* (the ready-to-eat paste made from *elubo*) in the present study.

Yam flour processing is similar among the West African countries, such as Nigeria, Benin and Ghana. This involves peeling dry yam tubers, sometimes slicing, blanching in hot water at a temperature between 40 and 60°C for up to three hours and steeping for about a day and sun drying. The parboiled, steeped and sun-dried product known as *gbodo* among the Yoruba speaking people of Nigeria is known as *elubo* when milled, and the finished (final) product made by stirring *elubo* into boiling water to make a thick paste is known as *amala* [12]. *Amala* is usually eaten with soup by consumers [13,14]. The main quality attributes of *amala* are colour, texture and taste [14]. Most consumers prefer a light brownish, elastic, nonsticky *amala* with a slightly sweet taste, while a slightly bitter taste is also tolerated [14,15,16].

Traditionally, sun-drying in the open being a low cost processing method is perhaps the most popular method employed in yam flour production. Sun drying as a method of food preservation, however, has some limitations including the lack of control on the drying process, unstable weather and food contamination among other undesirable characteristics [17]. On the other hand, hot air drying in a controlled environment in a set-up whereby moisture is removed from food materials by blowing heated air over the food materials with the aid of fans. The observed colour changes during the drying process is usually as a result of the physico-chemical changes in the product during the drying process [9].

Lactic acid fermentation is a commonly used method for preservation of plant materials as well as obtaining desirable sensory and nutritional properties to the product [18]. The present is focused on standardising 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.

2. MATERIALS AND METHODS

2.1 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.

The yam samples were washed, peeled, diced, soaked in water at 50°C for 24 hours, dried, milled to flour and then sieved, this was done according to the method described by Babajide et al. [19]. The flow chart used in processing the yam tubers into yam flour is shown in Chart 1. Microbial and proximate samples were taken for sample analyses within 24 hours of steeping.

2.2 Identification of Isolates

Microbiological analyses were conducted immediately after sampling. Sampling was done by agitating the steeped yam before sampling to ensure uniform mixing, then, to 1mL of the sampled solution 90 ml of sterile normal saline was added, vortexed and further diluted in a 10-fold dilution series. For Lactic acid bacteria, 0.1 ml of suitable dilutions of inocula were spread onto De Man Rogosa Sharpe (MRS) agar, plates were incubated anaerobically at 30 °C for 24 h in an anaerobic incubator (Surgical Medical England Model SM-80CH, uv). Representative dominant colonies were picked from the plates of the suitable dilutions and purified by repeated streaking onto nutrient agar. For lactic acid fungi, 0.1 ml of suitable dilutions of inocula were spread onto Potato Dextrose Agar (PDA). Eight hourly changes over a period of 24 h in the microbial population of the total viable lactic acid bacteria and fungi were determined using MRS agar and PDA respectively. Samples were enumerated by using appropriate sterile dilution and spread plate

methods eight hourly. For the identification of microbial isolates, the fungal plates were incubated at 25°C for 2-5 days, while the bacteria were incubated at 30°C for 24- 48 h. Three colonies for each morphological type was purified and maintained in the appropriate agar plates. Systematic morphological and biochemical tests were conducted according to [20,21], moreover, identification of bacterial isolates into species was done according to tests and descriptions are given in [22] and [23]. The fungal isolates were characterised by their cultural properties stained with cottonblue lactophenol solution and observed microscopically [24].

2.3 Organoleptic Analysis

For the sensory evaluation (colour, aroma and texture), the *amala* obtained on zero fermentation was poured into a container labelled 0 h, *amala* of 8 h of fermentation into container labelled 8th hour, *amala* of the 16th hour of fermentation into the container labelled 16th hour, and so on till the 24th hour. A panel of thirty individuals were invited for the sensory evaluation (organoleptic appeal) of odour, taste, appearance, pasting, texture and general acceptability. The samples in the container were presented to the evaluators at random. The evaluators were asked to award scores for each sample after observing the colour, aroma and texture of each sample. The products were ranked on a scale of 1-5; 1 – extremely dislike, 2- dislike, 3- neither like nor dislike, 4- like and 5- like extremely [25].

2.4 Oven Drying Versus Sun Drying

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

2.5 Experimental Design

Complete Randomized Design (CRD) with three replications was used to test if spontaneous fermentation of yam improves the organoleptic characteristics of *amala* made from sun or oven-dried yam. These characteristics include odour, taste, appearance, pasting, texture and general acceptability. The results of the three replicates were pooled and expressed as mean ± 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 = .05 using SPSS software version 21.0.

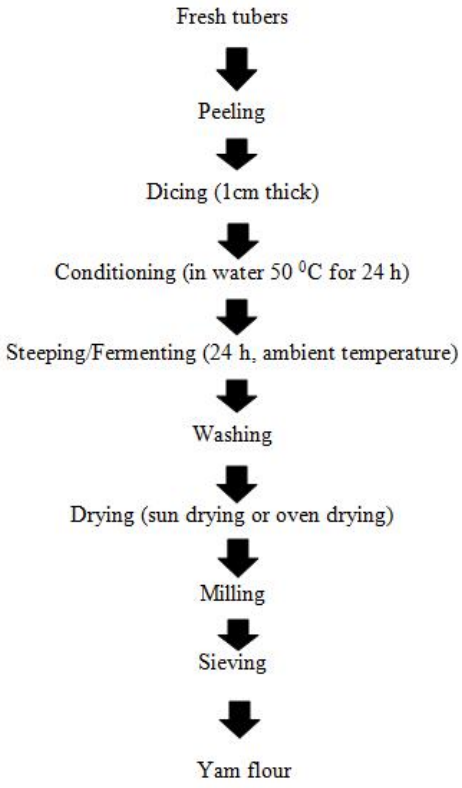

Chart 1. The flow chart for the production of yam flour

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Lactic acid Organisms Encountered in the Fermentation Process

As shown in the identification table (Table 1), the representative lactic acid bacterial isolates from the anaerobic culturing of samples from the fermentation of yam for the production of *elubo* were observed in only two of the four varieties that were evaluated, 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 grouping of representative isolates was based on the data on the cultural, gram staining and biochemical characteristics of the isolated organisms. 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).

Table 2 displays the basis of identification 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. The grouping of representative isolates was based on the data on the cultural, gram staining and biochemical characteristics of the isolated organisms. The results showed similarities among the predominant organisms for all the tested yam varieties. The four (4) organisms were identified as *Rhizopus spp, Aspergillus niger, Aspergillus flavus and Neurospora spp*. Similar organisms have been identified from the microflora of okra, *Abelmoschus esculentus*, a common vegetable food found in Nigeria and generally in the tropics [26]. In addition, similar organisms have been reported from the anaerobic fermentation of cassava, a staple root crop of the tropics from the same geographical location [27]. 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).

3.2 Succession of Organisms

The percentage frequency of isolation of the organisms encountered during the spontaneous fermentation process for *elubo* is shown in Table 3; 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 0.1×10^{-11} – 3.4×10^{-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 16th hour. Similar organisms were reported from the anaerobic fermentation of cassava at the same geographical location [27].

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 *amala* made from sun-dried yam variety TDr 95/ 19177 was the most preferred for odour, while *amala* from sun-dried TDr Alumaco was most preferred in terms of general acceptability. On the other hand, no statistical significant differences were found among the treatments when data in triplicates were compared using a one-way analysis of variance ANOVA at $p = .05$. The identified fermentation organisms isolated from the present study confirm similar studies that were done on yam fermentation, notably, the works of Achi et al. [28] and Babajide et al. [29]. Furthermore, these results 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*.

As shown in Fig. 1 and Table 3, the lactic acid bacteria, *Lactobacillus brevis* and lactic acid fungi, *Rhizopus spp, Aspergillus niger, Aspergillus flavus and Neurospora spp* occurred successively in the fermentation process and show a lot of promise and may be subsequently utilised in consistently improving the organoleptic

appeal of *amala*. This result 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 [28,30,25]. In previous reports, *L. plantarum* has been shown to predominate in cassava fermentation; this is indicative of its potential for development as starter cultures for yam flour (*elubo*) industrialisation. It is notable that success has been achieved in the use of lyophilised lactic acid bacteria as starter cultures for another indigenous African fermented food from cassava, namely, *gar*i production has been reported, where *L. plantarum* produced at low cost has been reportedly used in large-scale production of *gari* [31].

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. [29] where different species of *Aspergillus* and *Rhizopus* were identified from steeped yam fermentation.

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	+ No gas	+ Gas	- No gas	+ Gas	+ Gas	+ No gas	+ Gas	*Lactobacillus brevis*
TDr Pepa	+	Rod	+	+	+	- No gas	+ No gas	+ Gas	- No gas	+ Gas	+ Gas	+ No gas	+ Gas	*Lactobacillus brevis*

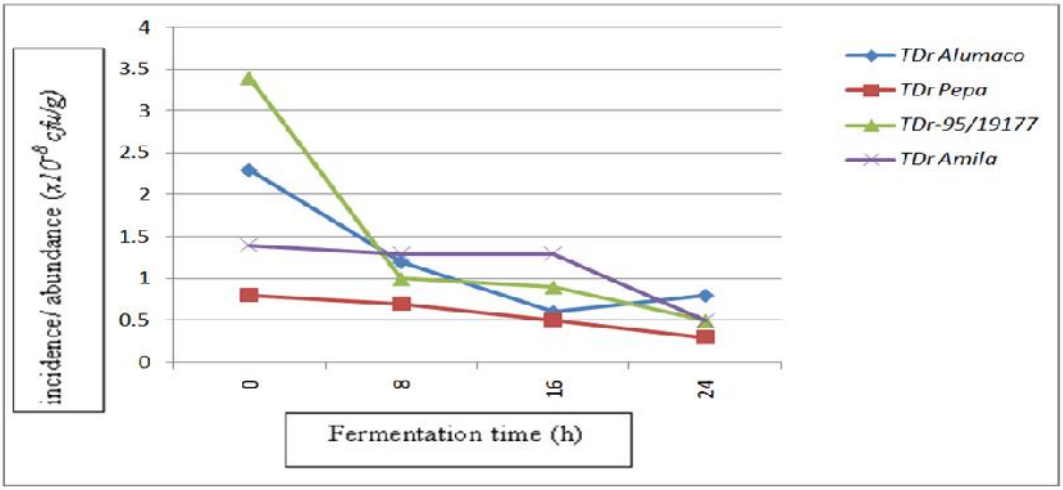

Fig. 1. A line graph tracking the typical incidence/ abundance (*x10^{-8} cfu/g)* of the lactic acid fungi from four varieties of yam (TDr Alumaco, TDr Pepa, TDr-95/19177 and TDr Amila) during *elubo* fermentation

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.	*Rhizopus spp*
2	Black spores with cream mycelia edges, same on reverse plate.	Hyphae is septate. Spore bearing.	*Aspergillus flavus*
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 biseriate, radiate, conidia in chains or detached and dispersed.	*Aspergillus niger*
4	Cream yeast-like spores, same on reverse plate.	Hyphae is non-septate. Conidiophores are branched and smooth. Head is radiated.	*Neurospora spp*

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

Yam variety	Alumaco				Pepa				Amila				TDr95/19177				Total
Fungal species	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	isolates
Rhizopus spp	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	3
Aspergillus flavus	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	12
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	14
Neurospora spp	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	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 $x10^{-11}$	1.2 $x10^{-11}$	0.6 $x10^{-11}$	0.8 $x10^{-11}$	0.8 $x10^{-8}$	0.7 $x10^{-8}$	0.5 $x10^{-8}$	0.3 $x10^{-8}$	3.4 $x10^{-8}$	1.0 $x10^{-8}$	0.9 $x10^{-8}$	0.1 $x10^{-8}$	1.4 $x10^{-11}$	1.3 $x10^{-11}$	1.3 $x10^{-11}$	0.1 $x10^{-11}$	

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

Table 4. Organopleptic 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)	4.0 (4.1)
TDr Amila	3.8 (3.5)	3.9 (4.0)	3.8 (3.5)	4.1 (4.3)	4.1 (4.0)	4.3 (4.2)
TDr Alumaco	3.8 (3.6)	4.1 (4.2)	4.3 (4.1)	4.5 (4.3)	4.4 (4.1)	4.5 (4.3)
TDr 95/ 19177	4.1 (3.8)	4.3 (4.1)	4.1 (4.3)	4.4 (4.4)	4.1 (4.3)	4.1 (4.3)

No statistical significant differences were observed when average organoleptic scoring data (in triplicates, n=30) were compared using a one-way analysis of variance ANOVA at p = .05

4. CONCLUSION

The versatility of the isolated lactic acid organisms identified from the present study is apparent from the results showing similarities in the type of microorganisms from the four varieties of yam evaluated in the present study. Moreover, the reports from literature showing that similar organisms have been isolated from cassava fermentation and other food products confirms that lactic acid bacteria and fungi have the ability to adapt to many different substrates [9,26,27]. In addition, the results underscore the importance of the prevailing fermentation microenvironment in determining the predominating organisms in the fermentation process and the organoleptic and nutritional characteristics of the final product. In conclusion, the present report documents the successful isolation of the lactic acid bacteria, *Lactobacillus brevis* and lactic acid fungi, *Rhizopus spp, Aspergillus niger, Aspergillus flavus and Neurospora spp* as fermentation organisms with potential to optimise the organoleptic and nutritional characteristics of *amala* in a reproducible manner peculiar to the test location being reported.

ACKNOWLEDGEMENT

The authors acknowledge and appreciate Drs. Peter Iluebbey and Norbert Maroya for providing the yam tubers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Okigbo RN. A review of biological control methods for post harvest yams *Dioscorea* spp.) in storage in South Eastern Nigeria. KMITL Sci J. 2004; 4(1):207-15.
2. Maroya NG, Asiedu R, Kumar PL, Lopez-Montes A, Orchard J, Ndiame F. YIIFSWA project description. Yam Improvement for Income and Food Security in West Africa, IITA, Ibadan, Nigeria; 2014.
3. Vernier P, Orkwor GC, Dossou AR. Studies on yam domestication and farmers' practices in Benin and Nigeria. Outlook on Agriculture. 2003;32(1):35-41.
4. Nweke FI, Ugwu BO, Asadu CL, Ay P. Production costs in the yam based cropping systems of southeastern Nigeria. RCMP research monograph/International Institute of Tropical Agriculture, Resource and Crop Management Program; No. 6.; 1991.
5. Ruel MT, Garrett J, Yosef S. Food security and nutrition: Growing cities, new challenges. International Food Policy Research Institute (IFPRI); 2017.
6. Asiedu R, Sartie A. Crops that feed the World 1. Yams. Food Security. 2010;2(4): 305-15.
7. Oluwatayo JI, Asiedu R, Adesiyan SO, Bamkefa BA, Bah ES, Dixon AG, Hamadina EI, Fifanou VG, Ousmane C, Gauthier B, Brice S. Studies on population build-up of *Scutellonema bradys* in *Dioscorea* species. African Journal of Root and Tuber Crops. 2011;9(1):24.

8. Akissoé N, Hounhouigan J, Mestres C, Nago M. How blanching and drying affect the colour and functional characteristics of yam (*Dioscorea cayenensis-rotundata*) flour. Food Chemistry. 2003;82(2):257-64.
9. Jimoh KO, Olurin TO, Aina JO. Effect of drying methods on the rheological characteristics and colour of yam flours. African Journal of Biotechnology. 2009; 8(10).
10. Achi OK. Quality attributes of fermented yam flour supplemented with processed soy flour. Plant Foods for Human Nutrition. 1999;54(2):151-8.
11. Adegunwa MO, Alamu EO, Omitogun LA. Effect of processing on the nutritional contents of yam and cocoyam tubers. Journal of Applied Biosciences. 2011;46: 3086-92.
12. Falade KO, Onyeoziri NF. Effects of cultivar and drying method on color, pasting and sensory attributes of instant yam (*Dioscorea rotundata*) flours. Food and Bioprocess Technology. 2012;5(3): 879-87.
13. Akingbala JO, Oguntimein GB, Sobande AO. Physico-chemical properties and acceptability of yam flour substituted with soy flour. Plant Foods for Human Nutrition. 1995;48(1):73-80.
14. Akissoé NH, Hounhouigan JD, Bricas N, Vernier P, Nago MC, Olorunda OA. Physical, chemical and sensory evaluation of dried yam (*Dioscorea rotundata*) tubers, flour and amala, a flour-drived product. Tropical Science. 2001;41(3):151-5.
15. Akissoé N, Hounhouigan J, Mestres C, Nago M. How blanching and drying affect the colour and functional characteristics of yam (*Dioscorea cayenensis-rotundata*) flour. Food Chemistry. 2003;82(2):257-64.
16. Mestres C, Dorthe S, Akissoé N, Hounhouigan JD. Prediction of sensorial properties (color and taste) of amala, a paste from yam chips flour of West Africa, through flour biochemical properties. Plant Foods for Human Nutrition. 2004;59(3):93-9.
17. Adejumo BA, Okundare RO, Afolayan OI, Balogun SA. Quality attributes of yam flour (Elubo) as affected by blanching water temperature and soaking time. Int J Eng Sci. 2013;2(1):216-21.
18. Di Cagno R, Coda R, De Angelis M, Gobbetti M. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiology. 2013;33(1):1-10.
19. Babajide JM, Oyewole OB, Obadina OA. An assessment of the microbiological safety of dry yam (gbodo) processed in South West Nigeria. African Journal of Biotechnology. 2006;5(2):157-61.
20. Schillinger U, Lücke FK. Identification of lactobacilli from meat and meat products. Food microbiology. 1987;4(3): 199-208.
21. Stiles ME, Holzapfel WH. Lactic acid bacteria of foods and their current taxonomy. International Journal of Food Microbiology. 1997;36(1):1-29.
22. Collins CH, Lyne PM, Grange JM, Falkinham JO. Counting methods. Collins and Lyne's Microbiological Methods. 1995; 8:144-55.
23. Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Whitman WB. (Eds.) Bergey's manual of systematic bacteriology: The firmicutes. Springer Science & Business Media. 2011;3.
24. Cheesebrough M. Medical laboratory manuals for tropical countries. Microbiology and Parasitology. 2005;209-235.
25. Aderiye BI, Ogunjobi AA. Fermentation of yam: microbiology and sensory evaluation of cooked fermented yam tissues. Plant Foods for Human Nutrition. 1998;52(1):49-54.
26. Fagbohun ED, Faleye OS. Effect of storage on chemical composition and mycoflora of okra (*Abelmoschus esculentus*). Int J Biosci. 2012;2(7):83-9.
27. Ayoade F, Paulina O, Kellanny S, Yeitarere A, Titilayo O, Scott O, Nicholas O, Abazuh UD, Tolulope A, Daramola GG, Onikepe F. The predominant lactic acid microorganisms and proximate composition of spontaneously fermented Gari and Fufu, Cassava Food Products. Annual Research & Review in Biology. 2018;26(2).
28. Achi OK, Akubor PI. Microbiological characterization of yam fermentation for 'Elubo' (yam flour) production. World Journal of Microbiology and Biotechnology. 2000;16(1):3-7.

29. Babajide JM, Maina S, Kiawa B, Skilton R. Identification of fungal isolates from steeped yam (Gbodo): Predominance of *Meyerozyma guilliermondii*. Food Science and Biotechnology. 2015;24(3): 1041-7.

30. Ige MT, Akintunde FO. Studies on the local techniques of yam flour production. International Journal of Food Science & Technology. 1981;16(3):303-11.

31. Lacerda ICA, Miranda RL, Borelli BM. Lactic acid bacteria and yeasts associated with spontaneous fermentations during the production of sour cassava starch in Brazil. Int J Food Microbiol. 2005;105(2):213–219.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/26401