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Nutritional enrichment of *Lafun*: An African fermented cassava food, using *Lactobacillus plantarum* and *Saccharomyces boulardii*

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Abstract
Lafun is one of the regularly consumed fermented cassava food in many parts of West Africa, though it is nutritionally inferior in terms of protein and mineral contents. In the present work, the possibility of nutritional enrichment of *lafun* by controlled fermentation of cassava root tuber using *Lactobacillus plantarum* and *Saccharomyces boulardii* was carried out. Three samples of *lafun* were produced using the two organisms as monoculture and mixed culture while the fourth sample that was obtained from spontaneous fermentation served as control. The pH and titratable acidity (TA) (mg lactic acid/g) of the fermenting roots were measured at 0th and 72nd hour of fermentation. Standard methods were used for the determination of proximate parameters such as crude protein, ash, lipids, crude fibre, moisture content and carbohydrate. All samples were analysed for phosphorus, calcium, magnesium, potassium, sodium, manganese, iron, copper, and zinc. The pH changes observed for *Lactobacillus plantarum* fermentation (LPF), *Saccharomyces boulardii* fermentation (SBF), mixed culture fermentation (MCF) and spontaneous fermentation (SF) were from 6.2 to 4.3, 6.2 - 5.3, 6.1 - 4.6, and 6.2 - 4.9 respectively while the TA increased from 0.6 in all samples to 9.9, 3.6, 8.4 and 7.0 in LPF, SBF, MCF, and SF respectively. *Lafun* sample obtained from SBF had significantly higher ($p < 0.01$) proximate values (protein: 1.70%; ash: 15.20%; crude fibre: 5.80%) than those obtained from SF (protein: 1.25%; ash: 12.42%; crude fibre: 1.41%), LPF (protein: 1.13%; ash: 2.26%; crude fibre: 3.14%), and MCF (protein: 1.60%; ash: 11.53%; crude fibre: 4.22%). Of all the four fermentation setups, SBF had the highest P (0.07%), Ca (0.80%), K (1.86%), Mn (10.39 ppm), Cu (4.42 ppm), and Zn(16.91 ppm) contents. This study shows a potential for nutritional improvement of *lafun* using *Saccharomyces boulardii* as starter culture.

Keywords: bio-fortification, cassava, fermentation, *Lafun*, *Saccharomyces boulardii*

Introduction

The flour called *Lafun* both in the Republic of Nigeria, and the Benin Republic, *Udaga* or *Kondowole* in Tanzania, *Cossettes* in Democratic Republic of the Congo, *Kokonte* in Ghana and Togo, and *Ikivunde* in Burundi (Abass *et al*., 2018) [1] is a fibrous powder. It is a good source of carbohydrate but has low protein content. It is traditionally produced by peeling and cutting fresh cassava roots into pieces followed by submerged and spontaneous fermentation for 2-5 days. During its fermentation process, various biochemical changes occur such as degradation of cyanogenic compounds, softening of the roots and formation of flavour compounds (Oyewole and Odunfa, 1992) [18]. However, just like other traditional fermented products, *Lafun* is spontaneously fermented in a cottage setting, and its dominant microbiota include *Lactobacillus fermentum, L. plantarum, Weissela confusa, Saccharomyces cerevisiae, Pichia scutulata, Kluyveomyces marxianus* and *Hanseniaspora guilliermondi* to mention but a few (Padonou *et al*., 2009) [19].

In spontaneous fermentation, the indigenous microorganisms compete for growth requirements which leave only the fittest to dominate the process. The dominating microorganisms however changes per time due to both exogenous and endogenous factors. Huch (née Kostinek) *et al*. (2008) [9] opined that there could be a high risk of failure of fermentation process where initiation of fermentation takes a longer time in a spontaneous process which then results in growth of spoilage microbes and poor product quality. Many researchers have therefore recommended the use of starter cultures in food fermentation based on food safety, protection, security, and quality among others.

The use of Lactic acid bacteria (LAB) to standardise the production of fermented food from cassava roots (Kostinek *et al*., 2007) [13] is fast becoming a keen interest. LAB show a lot of basic functional attributes for starters (Leroy and De Vuyst, 2004) [14]. Among these attributes are: 1. Producing antimicrobial substances that contribute to the safety of foods and immensely to the survival of human life; 2. Enhancing organoleptic and nutritional properties; 3. Reducing anti-nutritional factors; 4. Ability to realise fast acidification of fermented products thereby preserving them and 5. Contributing to the liberation of bioactive peptides that promote health (Proteolytic LAB) or hydrolysing starch and thus increasing the energy density of starchy food (Amylolytic LAB) (Kostinek *et al*., 2007) [13].

Yeasts have wide applications in protein production. Their biomass have been used either directly as a protein supplement or as part of fermentation by-product (Day and Morawicki, 2018) [4]. Oseni and Akindahunsi, (2011) [17] stated that fermentation could increase protein content of food through secretion of microbial proteins, accumulation of other nitrogenous microbial components such as chitin, and hydrolysis of peptides. Crude protein content of a food product could also increase when protein in the substrate is concentrated as carbohydrates are consumed by microorganisms (Day and Morawicki, 2018) [4].

The development of simple processing method that will increase the nutritional content of *Lafun* and improve its quality could increase consumers' acceptability thereby securing its existence in the food chain. This work was therefore carried out to investigate a possible nutritional enrichment of *Lafun* using *Lactobacillus plantarum* and *Saccharomyces boulardii* as starter microorganisms.

Materials and Methods

Collection and Preparation of Samples

Fresh cassava roots (TMS-IBA30572) were sourced from International Institute of Tropical Agriculture (IITA), Moniya, Ibadan, Nigeria. These cassava roots were harvested, collected in clean, sterile polythene bags, and processed (washed, peeled, sized, and washed) immediately. The processed roots were initially blanched for 2 min to reduce microbial loads and cooled under a UV light before fermentation.

Starter Cultures

Previously characterized and typed microorganisms (*Lactobacillus plantarum* and *Saccharomyces boulardii*) were obtained from the culture collection center of Biology Department, The Polytechnic, Ibadan, Nigeria. *Lactobacillus plantarum* strain was isolated from fermenting cassava product while *Saccharomyces boulardii* was isolated from lychee fruit that was bought from a multi-national Supermarket in Ibadan, Nigeria. The two microorganisms exhibited high linamarase and amylase activities, as well as tolerant to 2% bile salt. In addition, they lack gelatinase and haemolytic activities. Stock cultures of these microorganisms stored at - 40 $^{\circ}$C in MRS broth and yeast extract broth (Difco, Michigan, USA) respectively for *L. plantarum* and *S. boulardii*, containing 20% v/v glycerol were resuscitated by inoculating in MRS broth and yeast extract broth (Difco, Michigan, USA) at 33 $^{\circ}$C and 28 $^{\circ}$C respectively for 72 h.

Production of *Lafun* by Control and Spontaneous Fermentation

Three *Lafun* samples were produced using the selected strains both as monoculture and mixed culture. The set-up replaced the rudimentary equipment used in the traditional method with glassware as described by Omafuvbe *et al*. (2002). About 24 h old cultures of *L. plantarum* and *S. boulardii* were grown on MRS agar (37 ^{o}C ± 2 ^{o}C) and Yeast Extract agar (33 ^{o}C ± 2 ^{o}C) respectively for 24 h. A loopful of each of the culture was suspended into 10ml sterile 0.9% NaCl solution and diluted to give an absorbance of 0.03 at 540 nm in a spectrophotometer. The suspensions of each culture were mixed equally for the mixed culture. Then, 5000 µl of the culture solution was inoculated into 500 g blanched cassava that was steeped in 500 ml of sterile water in 2 L sterile conical flask. The inoculated cassava roots were fermented under anaerobic condition for lactic fermentation (37 ^{o}C ± 2 ^{o}C) and aerobic condition for yeast and mixed culture fermentation (33 ^{o}C ± 2 ^{o}C) for 72 h. In the spontaneous fermentation setup, fresh cassava roots were peeled and cut into pieces (about 5 cm x 2 cm x 2 cm) and washed. The cassava pieces were soaked in water for 3 days. At the end of 72 h, the samples were dried in an oven at 55^{o}C, milled, packed, and kept at -18 ^{o}C for subsequent analyses.

Measurement of pH

The pH value of the fermenting cassava roots was obtained using a pH meter (Mettler Toledo, AG8603 Switzerland model) whose electrode had been calibrated. Ten g of each milled sample was suspended in 90 ml of distilled water to obtain a one tenth dilution. The pH value of the slurry was obtained for all samples obtained at 0th and 72nd hour of fermentation.

Titratable Acidity

The method described by Ikenebomeh *et al*. (1986) [10] was employed to estimate the titratable acidity of the fermenting samples. Fifty microlitre aliquot of the ferment water was titrated with 0.1M NaOH with phenolphthaline as the end point indicator. Ten millilitres of decarbonated water was also titrated and the water titre subtracted from the sample titre. One millilitre of 0.1 N NaOH was taken as equivalent to 9.0 x 10^{-3}g lactic acid. Titratable acidity was calculated as milligram of lactic acid per gram of sample (mg g^{-1}).

Proximate Analysis of *Lafun* Samples

The determinations of moisture, ash, Crude protein, Carbohydrate, and Crude fibre contents were carried out according to the methods of AOAC (1995) [2].

Determination of Mineral Contents

The mineral (phosphorus, calcium, magnesium, potassium, sodium, manganese, iron, copper, and zinc.) analysis of the *Lafun* samples was carried out using Atomic Absorption Spectrophotometer (Analytikjena AG, Germany) according to the method of Hernandez *et al*. (2004) [8]. Five grammes of sample were placed in a previously weighed porcelain crucible. It was placed in a muffle furnace and ashed at 500˚C for two hours. The resulting white ash was weighed, dissolved in 3ml of concentrated nitric acid and diluted with distilled water in a 25ml calibrated flask. The solution was used to determine Fe, P, K and Ca. Standard stock solutions of Fe, P, K and Ca were prepared from AAS grade chemicals (Sigma, USA) by appropriate dilution.

Statistical Analysis

Data obtained were expressed as means ± standard deviation. Analysis of variance was carried out on the data obtained to determine the significance of differences. A two-tailed P value of less than 0.01 and 0.05 was considered to be statistically significant. Values that were significantly different were separated using the Duncan Multiple Range test using SPSS for Windows Version 17.0 statistical package.

Results and Discussion

Table 1 shows the pH and titratable acidity (TA) **(**mg lactic acid g^{-1}**)** of the fermenting cassava roots. The pH of the fermenting cassava roots at the beginning of fermentation was between 6.10 and 6.20. However, at the end of 72 h fermentation the pH of the fermented roots ranged between 4.3 and 5.3. The pH changes observed for *Lactobacillus plantarum* fermented (LPF), *Saccharomyces boulardii* fermented (SBF), mixed culture fermented (MCF) and spontaneous fermented (SF) *Lafun* were from 6.2 to 4.3, 6.2

- 5.3, 6.1 - 4.6, and 6.2 - 4.9 respectively. In another development, the TA increased from 0.6 in all samples to 9.9, 3.6, 8.4 and 7.0 in LPF, SBF, MCF, and SF *Lafun* respectively. In all the fermentation setups, *L. plantarum* brought about the highest level of acidification and *S. boulardii* the least. This is evident by the highest drop in pH value (6.2 – 4.3) and the highest value (9.9) of TA for the former and 6.2 – 5.3 and 3.6 for the latter respectively. The degree of acidification observed in the present study is comparable to the values reported for spontaneous fermentation of *Lafun*. Padonou *et al*. (2009) [19] reported a pH drop from 6.2 to 4.3 and TA increase from 0.42 to 1.23% (4.2 to 12.3 mg g^{-1}) in spontaneous fermentation of *Lafun* in the principal *Lafun* production area in Benin Republic.

Table 1: pH and titratable acidity **(**mg lactic acid **g^{-1})** of fermenting cassava roots

Sample	pH		Titratable acidity	
	0 h	72 h	0 h	72 h
LPF	6.20±0.02	4.30±0.03	0.60±0.03	9.90±0.00
SBF	6.20±0.01	5.30±0.02	0.60±0.00	3.60±0.01
MCF	6.10±0.01	4.60±0.02	0.60±0.01	8.40±0.00
SF	6.20±0.03	4.90±0.07	0.60±0.00	7.00±0.00

Values are means ± standard deviation (n=3)

Keys: LPF, s0ample fermented with *L. plantarum;* SBF, sample fermented using *S. boulardii;*
MCF, sample fermented with mixed cultures; SF, sample from spontaneous fermentation

The proximate compositions of the fermented *Lafun* produced through controlled and spontaneous fermentation in shown in table 2. *Lafun* sample obtained from SBF sample had significantly higher ($p < 0 .01$) proximate values (protein: 1.70%; ash: 15.20%; crude fibre: 5.80%) than those obtained from SF (protein: 1.25%; ash: 12.42%; crude fibre: 1.41%), LPF (protein: 1.13%; ash: 2.26%; crude fibre: 3.14%), and MCF (protein: 1.60%; ash: 11.53%; crude fibre: 4.22%) samples.The crude protein contents of the samples varied significantly ($p < 0.01$) with the highest value in *Lafun* sample fermented using *S. boulardii.* This may be as a result of yeasts proteins secretion (Oseni and Akindahunsi, 2011) [17] and (or) increase in growth and proliferation of yeasts in the form of single cell proteins (Boonnop *et al*., 2009) [3]. Researchers had noted that solid state fermentation with yeast and lactic acid bacteria (LAB) increased crude protein (CP) content of food materials with better values than was found in this work. Day and Morawicki (2018) [4] reported a crude protein increase from 9% to 27% in fermented grain sorghum using *S. cerevisiae* and *Lactobacillus amylovorus*- an amylolytic LAB. Similarly, earlier studies on solid-state fermentation of cassava pulp and cassava peels using *Aspergillus niger* and *Saccharomyces cerevisiae* increased the protein content to 7.91 – 9.04 and 14.14 – 16.74% respectively (Iyayi and Losel, 2001). Using *R. oryzae* and *S. cerevisiae*, Oboh and Oladunmoye (2007) [15] reported protein content of 8.8 – 12.6% in fermented cassava flour as Kolapo and Sanni (2009) [12] documented a protein content of 1.0 – 1.5% in naturally fermented gari. The dewatering after submerged fermentation during *Lafun* production would have been responsible for the low protein values obtained in the present study. In addition to protein enrichment, yeast fermented *Lafun* also showed a remarkably high ($p < 0.05$) ash and fibre contents (Table 2). The ash level after yeast fermentation could be corresponded to the level of incomplete utilisation of the nutrients present in the raw material by the yeasts (Eromosele *et al*., 2017) [6]. The lipid, moisture and carbohydrate contents of the samples are also significantly different ($p < 0.01$).

The mineral compositions of the unfermented and fermented cassava roots are shown in table 3. There is a significance difference of varying levels across the mineral elements of the *Lafun* samples. Nitrogen, calcium, sodium, and iron are significantly different ($p < 0.01$), with *Lafun* obtained through *S. boulardii* fermentation having the highest values of nitrogen and calcium. Also, *Lafun* samples obtained through *S. boulardii* fermentation had significantly ($p < 0.05$) highest content of magnesium, manganese, and zinc. Although the fermentation process does not significantly differentiate the phosphorus composition, there is a significant difference ($p < 0.01$) in the potassium and copper content. In this regard, *S. boulardii* fermented *Lafun* had the highest content of these minerals.

Table 2: Proximate composition of *Lafun* produced by lactic, yeast and spontaneous fermentation

	Proximate (%)					
Sample	**Protein**	**Ash**	**Lipid**	**Crude fibre**	**Moisture**	**CHO**
LPF	1.13±0.01	2.26±0.20	1.22±0.00	3.14±0.10	12.61±0.43	79.65
SBF	1.69±0.11	15.20±0.07	1.18±0.00	5.80±0.03	12.55±0.28	63.57
MCF	1.63±0.00	11.53±0.24	1.21±0.04	4.22±0.01	7.82±0.66	73.62
SF	1.25±0.01	12.42±0.26	1.33±0.00	1.41±0.09	15.68±0.26	67.91
UCR	1.16±0.02	1.73±0.09	0.58±0.07	2.19±0.11	15.92±0.02	78.42

Values are mean ± standard deviation (n=3)

Protein, lipids, moisture, and carbohydrate are statistically significant at 1% level but there is a statistical significance at 5% level for ash and crude fibre. LPF, sample fermented with *L. plantarum;* SBF, sample fermented using *S. boulardii;* MCF, sample fermented with mixed cultures; SF, sample from spontaneous fermentation; UCR, unfermented cassava root

Table 3: Mineral composition of *Lafun* produced by lactic, yeast and spontaneous fermentation

	Macrominerals (%)					Trace minerals (ppm)				
Sample	**N**	**P**	**Ca**	**Mg**	**K**	**Na**	**Mn**	**Fe**	**Cu**	**Zn**
LPF	0.18	0.02	0.78	0.02	0.22	25.18	04.37	07.36	1.07	07.34
SBF	0.27	0.07	0.80	0.05	1.86	25.69	10.39	11.57	4.42	16.91
MCF	0.26	0.06	0.78	0.04	1.85	30.13	08.90	07.36	2.19	13.97
SF	0.20	0.03	0.77	0.02	0.90	25.44	04.40	11.65	1.08	09.45
UCR	0.11	0.02	0.60	0.03	0.24	21.32	3.14	6.67	1.90	13.88

Values are mean of triplicate readings

N, Ca, Na, Fe are statistically significant at 1% level. Also, Mg, Mn and Zn showed a 5% level of significant difference, but K and Cu displayed a statistical difference of 10% level. LPF, sample fermented with *L. plantarum;* SBF, sample fermented using *S. boulardii;* MCF, sample fermented with mixed cultures; SF, sample from spontaneous fermentation; UCR, unfermented cassava root

Pranoto *et al*. (2013) [20] reported that fermentation increased magnesium, iron, calcium, and zinc content in some commonly consumed Indian fermented foods. These authors associated the increase in these minerals content with the decrease in the amount of phytates as fermentation progressed. In a bid to explain the mechanism responsible for the increase of mineral content of food sequel to fermentation, Day and Morawicki (2018) [4] opined that the increase in mineral content might be due to loss of dry matter during fermentation as microbes degrade carbohydrates and protein. Furthermore, Sripriya *et al*. (1997) [22] submitted that fermentation increases bioavailability of calcium, phosphorus, and iron likely due to degradation of oxalates and phytates that complex with minerals thereby reducing their bioavailability. In the present study, the mineral contents of the products are dependent on the starter cultures used during fermentation, and in this context, *S. boulardii* as a monoculture, appears to have a distinctive capability to bring about the mineral enrichment of *Lafun* samples.

Earlier studies have shown that *Saccharomyces boulardii* is the only yeast with clinical effects and the only yeast preparation with proven probiotic efficiency in double-blind studies (Sazawal *et al*., 2006) [21]. Van der aa Kühle and Jespersen (2003) [23] had reported that *S. boulardii* strains morphologically and physiologically could be characterized as *S. cerevisiae*. Results from the present study have indicated that *S. boulardii*, in addition to being used as a commercial probiotic, could also be used for bio-fortification of fermented cassava products such as *Lafun*. The recommended dietary allowances (RDAs) of the analyzed minerals for children aged 4 to 8 years and amount of nutrients obtainable from 100 g of *Saccharomyces boulardii* fermented (SBF) *Lafun* and spontaneous fermented (SF) *Lafun* are shown in table 4. In addition, the percentage daily values (%DV) of the RDA for each nutrient are also shown in the table. General guide to the interpretation of %DV stipulates that 5% DV or less of a nutrient per serving is considered low while 20% DV or more of a nutrient per serving is considered high (FDA, 2000). From the present study, it is evident that, SBF Lafun has high % DV for the following five nutrients: magnesium, potassium, sodium, manganese, and zinc while SF has high % DV for the following three nutrients: potassium, sodium, and manganese. In developing countries, where both hidden and obvious hungers are prevalent, SBF Lafun could serve as functional food of some sort.

Table 4: Recommended daily allowance (RDA) of nutrients for 4- to 8-year-old children and amount of nutrients obtainable from 100 g of *Saccharomyces boulardii* fermented *Lafun* (SBF) and spontaneous fermented (SF) *Lafun*

Nutrient	RDA	SBF	SF
Calcium (mg)	800	80 (10%)	77 (10%)
Phosphorus (mg)	500	70 (14%)	30 (6%)
Magnesium (mg)	130	50 (38%)	20 (15%)
Potassium (mg)	3.8	186 (4,894%)	90 (2,368%)
Sodium (mg)	1.2	2.6 (216%)	2.5 (208%)
Manganese (mg)	1.5	1.0 (67%)	0.4 (27%)
Iron (mg)	10	1.2 (12%)	1.2 (12%)
Copper (mg)	440	0.4 (0%)	0.1 (0%)
Zinc (mg)	5	1.7 (34%)	0.9 (18%)

Values in bracket are % daily value (%DV) of the RDA for each nutrient

Conclusion

Considering the results of fermentation in the present work, *S. boulardii* shows a good potential for improvement of some minerals content of *Lafun*. This demonstration of the possibility of *Lafun* bio-enrichment could increase the usage of cassava roots for more valuable products. Further works should concentrate on how to maximise protein enrichment of *Lafun* by complementing yeast fermentation with amyloglucosidase treatment, nitrogen (pepsin) supplementation or acid and alkali treatment that would enhance biosynthesis of amino acids by the fermenting organisms.

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