EVALUATION OF PHYSICO-CHEMICAL, NUTRITIONAL AND SENSORY PROPERTIES OF *Pennisetum glaucum* (MILLET) GRUEL FERMENTED USING *Lactobacillus brevis* AND *Sacchromyces cerevisiae*

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Astract
Lactobacillus brevis and *Sacchromyces cerevisiae* were isolated and identified from local yoghurt 'Kindirmo' and palm wine respectively. Single starter culture of *Lactobacillus brevis, Sacchromyces cerevisiae* and their combination were used during fermentation of millet gruel. The pH, temperature, titrable acidity, proximate composition and sensory evaluation of the gruel samples were analyzed during the 72hrs fermentation period. *Lactobacillus brevis* as starter culture exhibited the highest acid producing ability in the millet gruel, decreasing the pH of the gruel from 5.26±0.00 to 5.00±0.00 for millet gruel, with corresponding increase in the titrable acidity (TTA) from 0.03±0.00 to 0.60±0.00 for millet gruel during 72hr fermentation period. The effected changes in pH by *Sacchromyces cerevisiae* when used as starter culture ranged respectively from 5.26±0.00 to 5.4±0.00 for millet gruel and titrable acidity of 0.03±0.00 to 0.59±0.00 for millet gruel. When using combined starter cultures of *L.brevis* and *S. cerevisiae* the pH was5.26±0.00 to 5.07±0.00 and TTA of 0.05±0.00 to 0.18±0.00 for the millet gruel. The protein content ranged between 4.39±0.00 to 4.00±0.00 for millet gruel sample produced with the combined starter cultures of *L.brevis* and *S.cerevisiae*. 4.39±0.00 to 3.00±0.00 for millet gruel produced with *L. brevis* only. The protein, moisture, fat, carbohydrate content decreased during the fermentation period and increase in Ash and fiber content was observed. Sensory evaluation of the gruel using different starter cultures indicated the judges preferred the gruel Millet treated with both *Lactobacillus brevis* and *Sacchromyces cerevisiae*. It can be concluded that using both organisms in the fermentation of the gruel samples gave better organoleptic properties.

Keywords: Co – culture, Fermentation, Gruel, Organoleptic, Starter culture

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1. INTRODUCTION

Fermentation is a process that helps break down large organic molecules via the action of microorganisms into simpler ones. For example, yeast enzymes convert sugars and starches into alcohol, while proteins are converted to peptides/amino acids. The microbial or enzymatic actions on food ingredients tend to ferment food, leading to desirable biochemical changes responsible for the significant modification to the food. Fermentation is a natural way of improving vitamins, essential amino acids, anti-nutrients, proteins, food appearance, flavors and enhanced aroma. Fermentation also helps in the reduction of the energy needed for cooking as well as making a safer product (Nkhata et al; 2018 Xiang et al 2019). Therefore, microorganisms' activity plays a significant role in the fermentation of foods by showing changes in the foods' chemical and physical properties. Fermented foods have several advantages (Melini et al; 2019, Sanlier et al; 2019)

Fermentation is one of the oldest food preparation methods considered as safe and acceptable for improving the quality and safety of foods. Traditional fermentation technologies were based on a natural process whereby wet foodstuff undergoes microbial degradation and when the food is edible it was termed fermented where it was not considered as spoiled (Lee, 2009). Over the years, the fermentation process has developed such that organic substrates are now being converted into more desirable substances through the action of enzymes or microorganisms under controlled conditions to achieve several specific important functions.

Cereals are the major sources of energy and protein in the diets of most Africans. There are

various types of cereal which includes maize (*Zea mays*), sorghum (*Sorghum vulgare*) and millet (*Perinisetum specatum*).

Millets have excellent nutritional quality and are comparable to some commonly consumed cereals like wheat and rice (Ragaee *et al.,* 2006). It also offers several health benefits to consumers. These crops lack gluten and hence can be consumed by people suffering from celiac disease (Gabrovska *et al.,* 2002). Millet consumption can also lower glycemic response, which can be helpful for the treatment of type II diabetes (Choi *et al.,* 2005). Inclusion of millet in the human diet can also lower the risk of duodenal ulcers, anemia and constipation (Jayaraj *et al.,* 1980; Nambiar *et al.,* 2011). For patients suffering from allergic diseases such as atopic dermatitis, Japanese barnyard millet grains have been recommended to replace rice and wheat grains (Watanabe 1999). Dietary fibre content in pearl and finger millet was found to be higher than that in sorghum, wheat and rice (Kamath and Belavady 1980). Millets are also rich in phenolic acid and have high antioxidant activity (Chandrashekhar and Shahidi 2010). They are valuable sources of some essential minerals such as potassium, magnesium, calcium, iron and zinc (Ravindran 1991).

Gruel is a traditional lactic acid fermented starchy meal, made from cereals such as maize (*Zea mays*), millet *(Perinisetum specatum)* or sorghum (*Sorghum vulgare*). Akingbala *et al.,* (1981) ; Teniola and Odunfa, 2001; Sanni *et al.,* 2002). The porridge forms an integral part of the adult main meal in most African countries and plays a vital role in the nutrition of infants and young children as complementary food.

Gruel is a light usually thin, cooked cereal made by boiling meal, especially oatmeal, in water or milk the main forms of gruel include rice gruel, flour gruel and millet gruel. Though, its actual medical use is not proven, the importance of gruel as a form of sustenance has historically been considered for the sick and recently weaned children (Maguelonne, 2009). AIM

2. MATERIALS AND METHODS

This study was carried out at the Department of Microbiology, Bayero university kano. The grain millet used for this research was obtained from Dawanau market, Kano State, Nigeria. The grains were brought into the laboratory in clean polyethylene nylons for immediate use. The seeds were carefully freed from foreign materials as well as broken and shrunken seeds.

Laboratory preparation of Millet gruel

Using the method of john (2008). The grains were sterilized using 1% sodium hypochlorite for 10minutes, it was then drained out and washed sing sterile distilled water 3 times and steeped in sterile distilled water for 1 day.

After steeping for 1 day it was then wet milled and sieved to remove bran (Teniola and odunfa 2002). The fermentation was then set up by mixing the slurry with 1ml of the standardized inocula in a plastic container. It will be possible to visualize some changes like air bubbles from the metabolic activities of some of the fermented microorganisms in the fermentation process (patience, 2013). It was then kept in a sterilized safety cabinet for 3 days of fermentation.

Assessment of fermentation

The extent of fermentation under the various conditions was assessed (Oyewola and Odunfa, 1988). The fermenting medium was assessed at 0hr and then at 12hr interval for 3 days. The parameters used for the assessment include physiochemical, Sensory evaluation and proximate analysis.

Determination of physiochemical parameters during fermentation

Determination of pH

A pH meter was used for this purpose. It was used to measure the acidity and alkalinity of the gruel Samples. The electrode was rinsed with distilled water and immersed into the samples. The pH of the suspension was measured using pH meter at 0, 24, 48 and 72hrs (AOAC, 2002). All analyses were carried out in triplicate.

Determination of temperature

The temperature was determined by inserting a sterile thermometer into each of the samples at 0, 24, 48 and 72hrs of fermentation. The

mercury-in-glass thermometer was used (AOAC, 2000). All analyses were carried out in triplicate.

Determination of proximate composition

The method of AOAC (1990) was used for the determination of protein, fat, moisture, Ash, crude fiber and Carbohydrate of each of the samples before and during the fermentation at 0, 24, 48 and 72 hours interval. All analyses were carried out in triplicate

Total Titrable Acidity (TTA)

The TTA of fermenting medium (expressed as percentage lactic acid) was determined according to Amoa - Awua *et al.,* (2006) by titrating 10ml of the decanted homogenate samples used against 0.1 NaOH using a drop of phenolphthalein as indicator before and during the fermentation period.

Total LAB count during fermentation

The total mean of LAB specie during fermentation was obtain using pour plate technique by serial dilution using MRS agar. It was obtained four times during fermentation and storage. The isolate obtained where further identified using biochemical technique.

Sensory Evaluation

The sensory evaluation of gruel samples were carried out to determine the acceptability of the product. The product (gruel) was subjected to organoleptic assessment by a 5 member panel of each of millet and sorghum gruel. Each panelist was requested to taste the sample one after the other and to indicate the degree of likeness or preference for the sample on the questionnaire provided (David, 2005).

3. RESULTS AND DISCUSSION

Table 1 and 2 shows the biochemical characteristics and sugar fermentation of bacteria isolated from local yoghurt (kindirmo) and fungal isolate from palm wine. They were *Lactobacillus brevis* and *Sacchromyces cerevisiae.* Similar results were reported by W. Dib *et al.,* (2014), Who isolate *Lactobacillus brevis* from local yoghurt kindirmo and Nwakanma *et al.,* (2015) who isolated *Sacchromyces cerevisiae* from palm wine in some towns in Enugu.

Table 3 shows the pH, Temperature and titrable acidity of the isolate at 24hr during the fermentation period. The pH and titrable acidity were 5.26±0.00 and 0.03±0.00 for millet gruel respectively. After 72 hours, Significant decreased and corresponding increase in pH and titrable acidity in millet gruel samples were observed, which could be attributed to the activity of *Lactobacillusbrevis* producing acid primarily lactic acid which is in line with findings of Odunfa (1985) and Sanni (1988). The fermentation is characterized by reduction in pH and its corresponding increases in titratable acidity to improve the safety of the product and also give it better antimicrobial properties. Decrease in pH was as a result of increased hydrogen ion content, probably due to the microbial activity on the carbohydrate and other food nutrients to produce organic acids. This agrees with the reports of Adeyemi and Umar (1994) Ogunbanwo S T *et al.,* (2013).

Table 4a and 4b shows the proximate composition of the samples during the fermentation period. There was decreased in fat content as the fermentation period progressed although the decreased is more pronounced in MS (3.90-1.87). Probably due to the fact that cereal grains are in general low in fat content. The result are in agreement with the findings of Kazanas and fields (1981) Nutritional improvement of millet and sorghum fermentation. Decreased in carbohydrate is less pronounced in MM (44.00) is due to utilization of glucose by the microorganisms. Similar result has been reported for sorghum (El-Tinay et al., 1979; Kazanas and fields, 1981; Chavan et al., 1988). Changes in carbohydrates (lactose) may be attributed to breakdown of carbohydrate into fermentable sugars by the fermenting microorganisms and their enzymes. There was decreased in moisture content in millet gruel samples. Different factors affect moisture content of food products. The variation in moisture content might be attributed to treatments, which caused changes in other nutrient contents. The protein content was found to decreased as the fermentation period progressed .The decreased is less pronounced in M and MS (4.39-4.27), Sample ML, MM, are in

the same range (3.00-3.90).The decreased is due to fermentation. El-hag et al., (2002) also reported a decreased in protein in fermented pearl millet. The protein content was found to decreased as the fermentation period progressed .The decreased is more remarkable in M and MS (4.39-4.27), Sample ML, MM, are in the same range (3.00-3.90).The decreased is due to fermentation. El-hag et al., (2002) also reported a decreased in protein in fermented pearl millet. The crude fiber content increased as the fermentation period progressed.

Table 5 Present the counts of isolates on MRS, Which considered to be lactic acid bacteria. The mean counts ranged between (10.24±0.21 - 5.54±0.01) for millet gruel. Which is in close agreement with the finding of Okoronkwo (2014) Isolation and characterization of lactic acid bacteria involved in fermentation of millet and sorghum sold in Nkwo-Achara market, Abia state. Who reported the LAB count between 11.5-5.75 in millet gruel.

Table 6 presents the mean of overall acceptability of millet gruel using *Lactobacillus brevis, Saccharomyces cerevisiae* and mixture of the organisms at different intervals. It could be observed that from the results a significant decrease in overall acceptability in all samples at the end of fermentation (72 hours). At 24 hours, the overall acceptability of samples M, ML, MS and MM, were 7.8±0.2, 7.4±0.2, 5.6±0.4, 6.6±0.6 respectively. All the samples received likeness by the panelist at 24-48 hours with the exception of MS which has 80% likeness. Highest score (7.8±0.2) was recorded in Untreated millet gruel (M) at the end of 72 hours.

Table 1: Morphological and biochemical characterization of *Lactobacillus brevis* and *Sacchromyces cerevisiae*

Microscopic observation/biochemical test	**Characteristics**	
Colony morphology	Cream large clear colonies	Cream, flat, smooth and moist
Cell morphology	rod-shaped	globular shaped
Gram reaction	+	+
Catalase test	+	-
Endospore staining	-	-
Germ tube test	-	+
Urease test	+	-

S/N	*Lactobacillus brevis*	*Sacchromyces cerevisiae*
1.	Glucose	+
2.	Glycerol	-
3	Calcium 2-keto-gluconate	-
4	Arabinose	-
5	Xylose	-
6	Adonitol	-
7	Xylitol	-
8	Galactose	+
9	Inositol	-
10	Sorbitol	-
11	Methyl D-glucopyranoside	+
12	N-acetyl-glucosamine	-
13	Cellobiose	-
14	Lactose	-
15	Maltose	+
16	Saccharose	+
17	Trehalose	+
18	Melezitose	+
19	Raffinose	+
20	Identified isolate	*S.cerevisiae*

Table 2: Sugar fermentation pattern of the isolate (API 50)

S/N		
1	Glycerol	-
2	Erythritol	-
3	D-Arabinose	-
4	L-Arabinose	+
5	Ribose	+
6	D-Xylose	+
7	L-Xylose	-
8	Adonitol	-
9	β-Methyl-xyloside	-
10	Galactose	+
11	D-Glucose	+
12	D-Fructose	+
13	D-Mannose	+
14	L-Sorbose	-
15	Rhamnose	-
16	Dulcitol	-
17	Inositol	-
18	Mannitol	+
19	Sorbitol	-
20	αMethyl Mannoside	-
21	α-Methyl-D-Glucoside	-
22	*N*-Acetylglucosamine	+
23	Amygdaline	+
24	Arbutine	+
25	Esculine	+
26	Salicine	+
27	Cellobiose	+
28	Maltose	+
29	Lactose	+
30	Melibiose	+
31	Saccharose	+
32	Trehalose	+
33	Inulin	+
34	Melezitose	-
35	D-Raffinose	+
36	Amidon	-
37	Glycogene	-
38	Xylitol	-
39	β-Gentiobiose	+
40	D-Turanose	-
41	D-Lyxose	-
42	D-Tagatose	-
43	D-Fucose	-
44	L-Fucose	-
45	D-Arabitol	+
46	L-Arabitol	-
47	Gluconate	+
48	2 keto-gluconate	-
49	5 keto-gluconate	-
	Identified isolates	*Lb brevis*

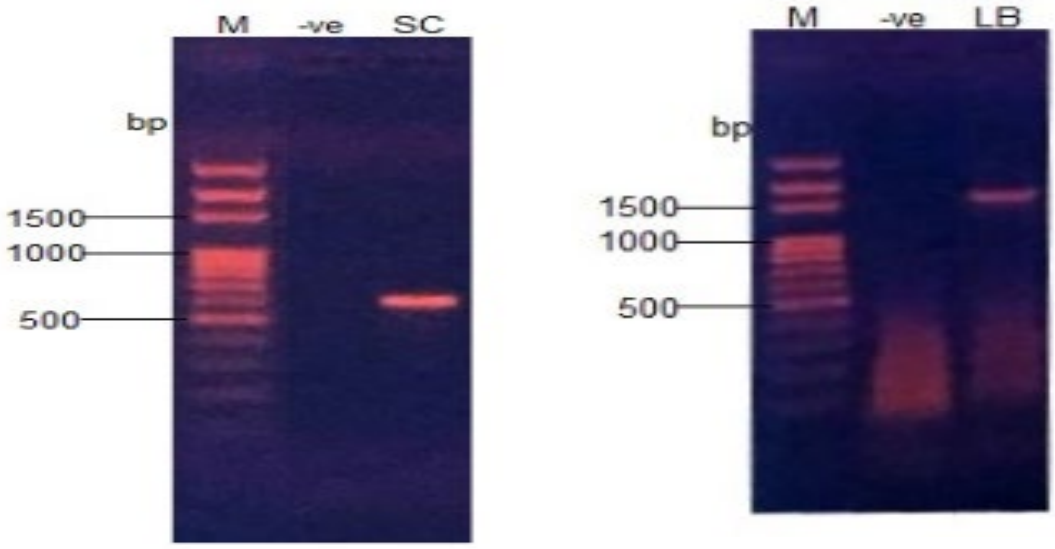

Fig. 1. Electrophoretograms showing the amplicons of a) *Lactobacillus brevis* and b) *Saccharomyces cerevisiae* isolates.
Key: M = ladder, -ve = negative, LB = *L.brevis* and SC = *S. cerevisiae* amplicon .

Table 3: Changes in Temperature, pH and Titratable acidity during fermentation of millet at 24hr intervals.

Parameter	Time	M	ML	MS	MM	Analysis
Temp	(Hr)					
	0	30±0.00	30±0.00	30±0.00	30±0.00	
	24	30±0.00	30±0.00	31±0.00	30±0.00	
	48	30±0.00	30±0.00	30±0.00	31±0.00	
	72	30±0.00	29±0.00	29±0.00	29±0.00	NSD
pH						
	0	5.26±0.00	5.26±0.00	5.26±0.00	5.26±0.00	
	24	5.16±0.00	5.15±0.00	5.4±0.00	5.41±0.01	
	48	5.13±0.00	5.03±0.00	5.4±0.00	5.4±0.00	
	72	5.12±0.00	5.00±0.00	5.4±0.00	5.07±0.00	SD
TTA						
	0	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	
	24	0.48±0.00	0.53±0.00	0.47±0.00	0.48±0.00	
	48	0.59±0.00	0.59±0.00	0.50±0.00	0.59±0.00	
	72	0.65±0.00	0.60±0.00	0.53±0.00	0.59±0.00	NSD

KEY: M=Untreated (Control), ML= Millet gruel with *Lactobacillus brevis,* MS= Millet gruel with *Sacchromyces cerevisiae,* MM=Millet gruel with mixture of *L. brevis* and *S. cerevisiae,* SD = Significant Difference and NSD = Not significant Difference

Table 4a: Change in Ash, Carbohydrates and fat contents during fermentation of millet gruel at 24hrs intervals

Parameters (%)	Time (hrs)	M	ML	MS	MM	analysis
Ash content	**0**	0.13±0.00	0.13±0.00	0.13±0.00	0.13±0.00	
	24	0.15±0.00	0.18±0.00	0.27±0.00	0.18±0.00	
	48	0.29±0.00	0.08±0.00	0.27±0.00	0.19±0.00	
	72	0.34±0.00	0.09±0.00	0.20±0.00	0.18±0.00	SD
C/content	**0**	45.90±0.00	45.90±0.00	45.90±0.00	45.90±0.00	
	24	41.07±0.00	48.51±0.00	47.50±0.00	48.90±0.00	
	48	41.00±0.00	43.02±0.00	49.00±0.00	41.30±0.00	
	72	43.30±0.00	44.29±0.00	49.35±0.00	44.00±0.00	NSD
Fat content	**0**	3.90±0.00	3.90±0.00	3.90±0.00	3.90±0.00	

24	1.15±0.00	1.66±0.00	1.07±0.00	1.29±0.00	
48	2.18±0.00	2.26±0.00	1.57±0.00	2.92±0.00	
72	2.09±0.00	2.52±0.00	1.87±0.00	2.80±0.00	SD

KEY: M=Untreated (Control), ML= Millet gruel with *Lactobacillus brevis,* MS= Millet gruel with *Sacchromyces cerevisiae,* MM= Millet gruel with mixture of *L. brevis* and *S. cerevisiae,* SD = Significant Difference and NSD = Not significant Difference

Table 4b: the change in moisture, crude fiber and crude protein contents of millet gruel at 24hours interval

Parameters (%)	Time (hrs)	M	ML	MS	MM	Analysis
Moisture	0	42.13±0.00	42.13±0.00	42.13±0.00	42.13±0.00	
Content	24	41.48±0.00	38.93±0.00	40.25±0.00	40.75±0.00	
	48	42.45±0.00	39.80±0.00	39.81±0.00	40.08±0.00	
	72	41.07±0.00	39.18±0.00	39.89±0.00	39.00±0.00	SD
Fiber content	0	3.57±0.00	3.57±0.00	3.57±0.00	3.57±0.00	
	24	3.76±0.00	6.50±0.00	7.21±0.00	5.02±0.00	
	48	9.83±0.00	10.87±0.00	5.69±0.00	11.34±0.00	
	72	9.00±0.00	10.89±0.00	4.61±0.00	10.00±0.00	SD
Crude protein	0	4.39±0.00	4.39±0.00	4.39±0.00	4.39±0.00	
	24	4.39±0.00	4.21±0.00	3.71±0.00	3.79±0.00	
	48	4.29±0.00	3.99±0.00	4.01±0.00	4.16±0.00	
	72	4.27±0.00	3.00±0.00	4.27±0.00	4.00±0.00	SD

KEY: M=Untreated (Control), ML= Millet gruel with *Lactobacillus brevis,* MS= Millet gruel with *Sacchromyces cerevisiae,* MM= Millet gruel with mixture of *L. brevis* and *S. cerevisiae,* and SD = Significant Difference.

Table 5: Changes in LAB count during the fermentation of gruel

Time(days)	M	ML	MS	MM	Analysis
0	10.24±0.21	10.24±0.21	10.24±0.21	10.24±0.21	
24	6.58±0.00	6.59±0.00	6.31±0.14	6.25±0.00	
48	5.60±0.01	6.38±0.00	6.08±0.00	6.17±0.01	
72	5.54±0.01	6.25±0.00	6.00±0.00	6.04±0.01	NSD

KEY: M=Untreated (Control), ML= Millet gruel with *Lactobacillus brevis,* MS= Millet gruel with *Sacchromyces cerevisiae,* MM= Millet gruel with mixture of *L. brevis* and *S. cerevisiae* and NSD = Not significant Difference

Table 6: The mean sensory scores (overall acceptability) of millet gruel and using *Lactobacillus brevis, Sacchromyces cerevisiae* and mixture of the two organisms at different storage interval.

Hour	**M**	**ML**	**MS**	**MM**	**Analysis**
0	8.2±0.8(80)	8.2±0.8(80)	8.2±0.8(80)	8.2±0.8(80)	
24	7.8±0.2(80)	6.8±0.2(80)	7.4±0.4(100)	6.4±0.6(80)	
48	7.0±0.4(100)	8.2±0.2(100)	6.0±0.3(60)	8.4±0.2(100)	
72	4.8±0.2(100)	4.2±0.2(100)	3.2±0.2(100)	4.4±0.2(100)	NSD

Key: M=Untreated (Control), ML= Millet gruel with *lactobacillus brevis,* MS= Millet gruel with *S. cerevisiae,* MM= Millet gruel with mixture of *L. brevis* and *S. cerevisiae*, figures enclosed in bracket represented the percentage of likeness/dislikeness and NSD = Not Significant difference.

4. CONCLUSION

In the present study, the isolation of *Lactobacillus brevis* from *kindrmo* and *Sacchromyces cerevisiae* from palm wine was carried out. The fermentation of millet gruel was characterized by a decrease in the pH from 6.84 to 5.00 and the corresponding increase in the titrable acidity from 0.03 to 0.60 which was observed throughout the period of fermentation. There was decrease in moisture content in millet gruel samples from 42.13- 39.00, carbohydrate content decreases in all the gruel samples from 46.15 to 43.25, fat content decreases from 3.90 to 0.26, protein contents decreased from 6.06 to 3.00 during the fermentation period, with increase recorded in ash content from 0.48 to 0.70 and crude fiber content also increase from 3.57 to 10.89. The sensory properties of the gruel using different starter cultures indicated

the judges preferred both millet and sorghum gruels treated with both *Lactobacillus brevis* and *Sacchromyces cerevisiae* throughout the fermentation period.

Recommendations

1. There is a need for further studies on the usage of different starter cultures, both singly or in combination with different densities, for traditional fermented foods, with a view to improve the shelf life, nutritional values and safety of fermented foods.
2. There is a need to optimize a standard modification in the fermentation process such as steeping time and milling time

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