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Effect of fermentation on proximate composition, physicochemical and microbial characteristics of pearl millet (*Pennisetum glaucum* (L.) R. Br.) and Acha (*Digitaria exilis* (Kippist) Stapf) flour blends

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The objective of this study was to determine the effect of spontaneous fermentation on proximate composition, physicochemical and microbial characteristics of sprouted millet-acha blends with the aim of producing fermented flour blends for traditional beverage. Pearl millet and acha grains were sprouted, milled and blended into different ratios. The blends were mixed with water, fermented for 72 h and dried in an air-oven. Proximate composition, pH, titratable acidity and microbial profile were determined using suitable methods. Moisture contents ranged from 5.60 to 5.94% and 10.44 to 13.33% for unfermented and fermented flour blends, respectively. Increase in pearl millet proportions significantly decreased (p ≤ 0.05) crude fat, ash, fibre and protein contents of the blends. In contrast, carbohydrate contents increased with increase in pearl millet proportion. Fermentation decreased significantly (p ≤ 0.05) the fat, fibre and carbohydrate contents of the flour blends; whereas ash and protein contents were significantly increased (p ≤ 0.05) with fermentation. pH values decreased with simultaneous increase in titratable acidity during fermentation. A total of 13 bacteria were isolated during fermentation, namely, *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus leichmonnii, Lactobacillus cellobiosus, Lactobacillus casei, Pediococcus* sp., *Streptococcus thermophilus, Micrococcus luteus, Bacillus subtillis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Proteus vulgaris*. Fermentation has a potential to improve protein and mineral contents of pearl millet-acha flour blends for beverage preparation.

Key words: Bacteria, beverage, fermentation, pearl millet, acha, flour, physicochemical, microbial characteristic.

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is considered as one of the most important drought tolerant crops of the tropical and subtropical regions in the world (Zakari et al., 2010). It is cultivated on more than 28. million ha of land in the semi-arid tropical regions of Asia (USDA, 2005). Nigeria is reported to produce about 6.7

million tons of pearl millet on 5.8 million hectares (USDA, 2005). Pearl millet contains about 1.6 to 2.4% ash, 2.6 to 4.0% crude fibre, 2.7 to 7.1% fat, 8.5 to 15.1% crude protein, 58 to 70% carbohydrate and 354 to 796 mg/g phytic acid (Abdalla et al., 1998). It is rich in minerals, such as calcium (10 to 80 mg/g), magnesium (180 to 270 mg/g) and phosphorus (450 to 990 mg/g) (Abdalla et al., 1998). Pearl millet also contains, potassium (70 to 110 µg/g) sodium (4 to 13 µg/g), zinc (53 to 70 µg/g), manganese (18 to 23 µg/g), copper (10 to 18 µg/g) and iron (70 to 180 µg/g) (Abdalla et al., 1998). In Nigeria and other parts of West Africa, pearl millet is used traditionally to produce fermented cereal based porridge ("Akamu" or "Ogi") (Zakari et al., 2010) and indigenous fermented beverage, such as "kunu-zaki" (Oluwajoba et al., 2013).

Acha (*Digitaria exilis* (Kippist) Stapf), also called "fonio" or "hungry rice" is reported as the oldest African cereal. It is an indigenous annual crop in West Africa (Chukwu and Abdul-kadir, 2008). Acha is reported to supply food to about 3 to 4 million people (Jideani, 1990; NRC, 1996). Acha contains about 1.6 to 3.6% ash, 0.48 to 1.90% crude fibre (Irving and Jideani, 1997; Chukwu and Abdul-kadir, 2008), 2.1 to 2.5% fat, 6.9 to 10.6% crude protein (Chukwu and Abdul-kadir, 2008; Irving and Jideani, 1997; Jideani, 1999) and 81.8 to 87.8% carbohydrate (Irving and Jideani, 1997; Jideani, 1999). It also contains minerals, such as calcium, magnesium, phosphorus, potassium, sodium, manganese, iron, zinc and copper (Chukwu and Abdul-kadir, 2008). Acha finds its application in traditional foods, such as porridge, couscous, alcoholic and non-alcoholic beverages (Jideani, 1999).

The use of acha and millet composite grains in the preparation of traditional fermented beverage (kunu-zaki) has been reported (Oluwajoba et al., 2013). However, in the study by these authors, proximate composition of the acha-millet grains composites were not reported, instead, the proximate composition of the kunu-zaki produced was reported. The present research sought to formulate pearl millet-acha flour blends for possible utilization in traditional beverage production. The objective of this study was to evaluate the effect of fermentation on the proximate composition, physicochemical and microbial characteristics of pearl millet and acha flour blends.

MATERIALS AND METHODS

Source of materials

Dry pearl millet (*P. glaucum* (L.) R. Br) and acha (*Digitaria exilis* (Kippist) Stapf) grains were purchased from a local market in Akure, Nigeria. The samples were transported to the laboratory in clean low density polyethylene bags.

Preparation of samples

Stones and other grits were sorted out from the grains, after which they were separately steeped in water for 5 h. The grains were then drained and separately spread on a clean tray and allowed to sprout at 25 ± 2°C for 24 h. The sprout grains were separately milled into flour using Hammer Mill (Brook Crompton, Huddersfield, England). The sprout pearl millet flour and sprout acha flour were mixed in sterile containers with appropriate labels in the following proportions (in grams): 200:100 (2:1) pearl millet to acha (A), 225:75 (3:1) pearl millet to acha (B), 240:60 (4:1) pearl millet to acha (C), 250:50 (5:1) pearl millet to acha (D) and 300:0 pearl millet to acha (E). Each of the samples was mixed with 1000 ml distilled water. The containers with the mixtures were covered, taped at the edges and the contents fermented for 72 h (Figure 1). At the end of fermentation, samples were dried in an air-oven at 65°C for 24 h and then packaged in low density polyethylene pouches and stored at 8°C prior to analyses. The fermentation process was carried out in three batches.

Proximate composition

The moisture, crude protein (N x 6.25), crude fibre, crude fat and total ash contents of samples were analysed before and after 72 hrs of fermentation using the method described by Association of Official Analytical Chemists' (AOAC, 1990) approved methods 925.10, 920.87, 920.86, 920.39 and 923.03 respectively. Total carbohydrate content of the samples was calculated by difference method (subtracting the sum of percent moisture, crude protein, crude fibre, crude fat, and ash from 100%).
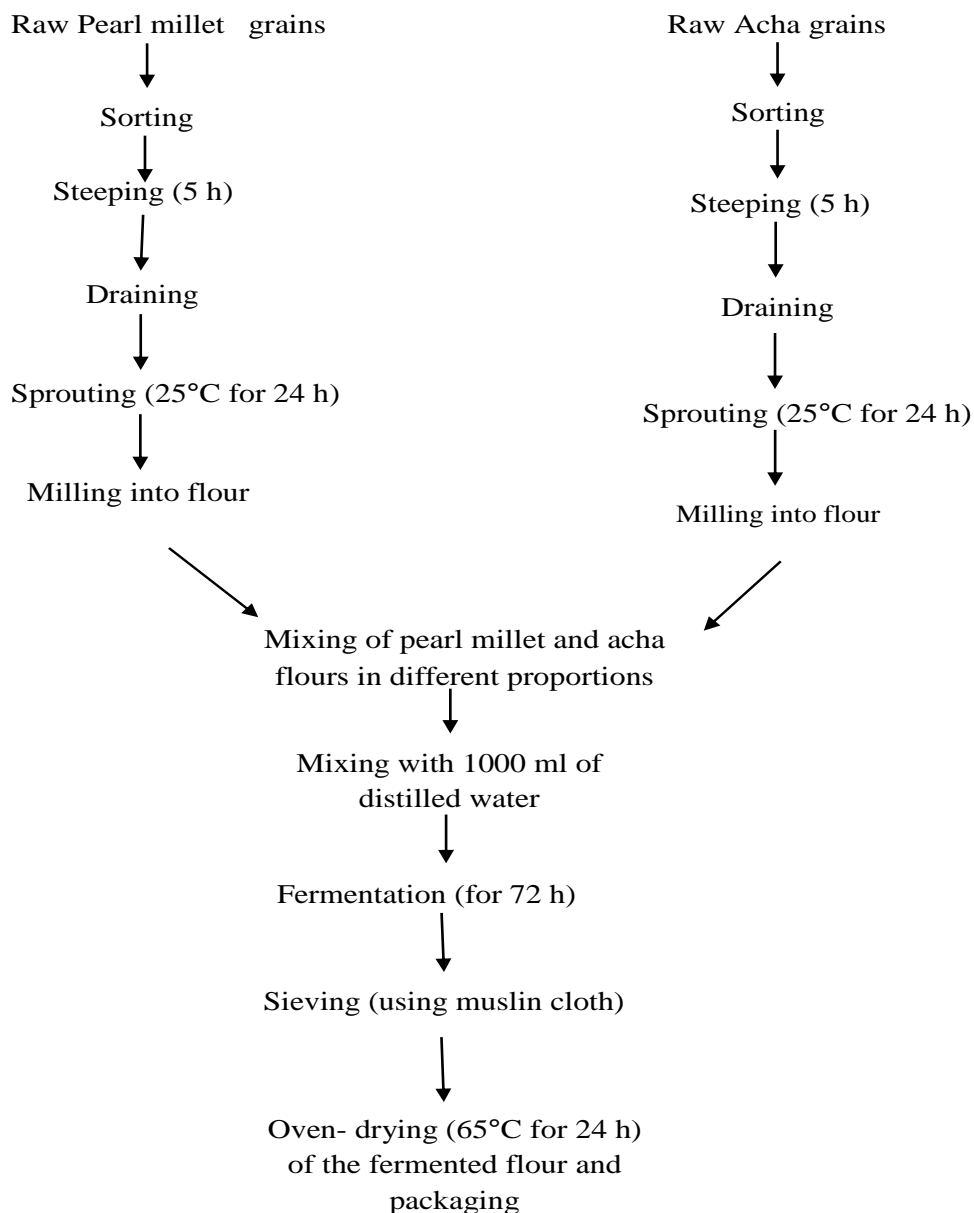
Physico-chemical properties

pH and titratable acidity

The method described by AOAC (1990) was used to determine pH and titratable acidity of the fermenting medium. Samples were taken every 24 h during the fermentation period according to the procedure described by Fayemi and Ojokoh (2014). The pH of the samples was determined using an Orion pH meter (Model 310, Orion Research Inc., Beverly, MA) equipped with glass electrode. The titratable acidity (TTA) was determined by titrating 10 ml of thoroughly mixed sample against 0.1 M NaOH using phenolphthalein as an indicator. Values obtained were expressed as percent lactic acid. All analyses were carried out in triplicate.

Microbial characteristics

The microbial profile of the raw and fermenting (at 24 h interval) blend samples were determined at 24 h interval. The changes in microbial population (cfu/g) of the total aerobic bacteria were determined using nutrient agar (NA) (Merck, Darmstadt, Germany) while De Man, Rogosa and Sharpe (MRS), (Merck) and M17 agar media (Oxoid, Basingstoke, Hampshire, England, UK) was used for the isolation of lactic acid bacteria (LAB). Four different colonies were randomly picked following visual assessment from the highest dilution factor of MRS and M17 agar plates to determine the dominant bacteria during the fermentation of the blends. Samples were analysed by homogenizing 1 g of the fermenting blend with 9 ml sterile 0.1% buffered peptone water (BPW) (Merck) followed by appropriate dilutions, spread plating and incubation at required temperatures. The NA agar plates were incubated at 37°C for 24 h while MRS agar plates were incubated anaerobically using anaerobic jar together with anaerocult system (Merck) at 37°C for 48 h. Colonies were selected randomly and purified before biochemical identification of the bacterial isolates (Collins et al., 1989; Fayemi and Ojokoh, 2014).

Raw Pearl millet grains

↓

Sorting

↓

Steeping (5 h)

↓

Draining

↓

Sprouting (25°C for 24 h)

↓

Milling into flour

Raw Acha grains

↓

Sorting

↓

Steeping (5 h)

↓

Draining

↓

Sprouting (25°C for 24 h)

↓

Milling into flour

↘   ↙

Mixing of pearl millet and acha
flours in different proportions

↓

Mixing with 1000 ml of
distilled water

↓

Fermentation (for 72 h)

↓

Sieving (using muslin cloth)

↓

Oven- drying (65°C for 24 h)
of the fermented flour and
packaging

Figure 1. Flow chart showing the preparation of fermented pearl millet- acha flour blends.

Statistical analyses

Analysis of variance (ANOVA) was performed on the data at $p \leq 0.05$ using MINITAB statistical software (Minitab® Release 14.13, Minitab Inc., USA). Significant means were separated using the least significant difference (LSD) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of fermented and unfermented pearl millet-acha flour blends is shown in

Table 1. Moisture contents of flour blends before fermentation (unfermented blends) ranged from 5.60 to 5.94%. Unfermented pearl millet flour (control, E) had significant ($p \leq 0.05$) low moisture content compared to flour blends and acha flour. After fermentation and drying, the flour blends had moisture contents ranging from 10.44 to 13.23% (Table 1). The moisture contents of all the samples were significantly ($p \leq 0.05$) different from each other. It could be seen from the results that moisture content of fermented pearl-millet flour blends were significantly higher than the unfermented samples. This could be attributed to the temperature and the duration of drying of the fermented samples. Moisture

Table 1. Proximate composition of fermented and unfermented sprout pearl millet-acha flour blends.

Proximate (%)	A		B		C		D		E		Acha*
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented
Moisture	$5.94^{Cb}\pm 0.03$	$13.23^{g}\pm 0.10$	$5.88^{Bb}\pm 0.00$	$12.78^{f} \pm 0.03$	$5.88^{Bb}\pm 0.01$	$11.89^{e}\pm 0.00$	$5.85^{Bb}\pm 0.01$	$11.26^{d}\pm 0.06$	$5.60^{Aa} \pm 0.00$	$10.44^{c} \pm 0.08$	$7.21^{D} \pm 0.01$
Fat	$7.31^{Dg}\pm 0.01$	$6.27^{f} \pm 0.03$	$6.22^{Ce}\pm 0.00$	$6.11^{d}\pm 0.01$	$6.18^{Ce}\pm 0.01$	$5.95^{c}\pm 0.00$	$6.10^{Bd}\pm 0.00$	$5.87^{b}\pm 0.04$	$5.94^{Ac}\pm 0.03$	$5.31^{a}\pm 0.00$	$8.40^{E} \pm 0.06$
Ash	$1.96^{Dc}\pm 0.01$	$3.68^{d} \pm 0.03$	$1.90^{Cbc}\pm 0.01$	$4.83^{e} \pm 0.00$	$1.84^{Bb}\pm 0.00$	$4.91^{f}\pm 0.03$	$1.69^{Aa}\pm 0.01$	$4.94^{fg}\pm 0.01$	$1.67^{Aa}\pm 0.04$	$5.09^{g} \pm 0.07$	$2.22^{E} \pm 0.00$
Fibre	$2.28^{Dh}\pm 0.01$	$0.65^{a} \pm 0.04$	$2.20^{Cg}\pm 0.01$	$0.79^{b} \pm 0.01$	$2.14^{Bf} \pm 0.00$	$0.93^{c} \pm 0.04$	$2.10^{Bf} \pm 0.00$	$1.39^{d} \pm 0.01$	$1.97^{Ae}\pm 0.03$	$1.43^{d} \pm 0.00$	$2.80^{E} \pm 0.03$
Protein	$11.52^{Ee}\pm 0.03$	$15.07^{f} \pm 0.10$	$11.00^{Dd}\pm 0.00$	$15.76^{g}\pm 0.14$	$9.40^{Cc} \pm 0.08$	$18.52^{i} \pm 0.00$	$9.10^{Bb} \pm 0.14$	$17.43^{h}\pm 0.15$	$8.00^{Aa} \pm 0.00$	$9.52^{c} \pm 0.03$	$20.15^{F} \pm 0.07$
Carbohydrate	$70.99^{Be}\pm 0.28$	$61.10^{c}\pm 0.14$	$72.80^{Cf}\pm 1.13$	$59.73^{bc}\pm 1.03$	$74.56^{Dg}\pm 0.79$	$57.80^{a} \pm 0.99$	$75.16^{DEg}\pm .23$	$59.11^{ab}\pm 0.16$	$76.82^{Eh}\pm 0.88$	$68.30^{d} \pm 0.28$	$59.22^{A}\pm 0.31$

Values are means ± standard deviation of duplicates. Values in the same row with different subscripts and or superscripts are significantly different at p < 0.05. Subscripts A – F compares all unfermented samples. * Acha was not fermented. A = 2:1 Pearl millet to acha blend, B = 3:1 Pearl millet to acha blend, C = 4:1 Pearl millet to acha blend, D = 5:1 Pearl millet to acha blend, E = 100 % Pearl millet (Control).

content values ranging from 10.16 to 10.33% and 9.25 to 10.35% have been reported for acha and fermented acha, respectively (Echendu et al., 2009). Zakari et al. (2010) reported moisture content values ranging from 12.0 to 12.9% have been reported for pearl millet-bambara groundnut blend flours.

Crude fat content of the unfermented pearl millet-acha flour blends ranged from 5.94 to 7.31% (Table 1). The fat content of acha flour was significantly (p ≤ 0.05) higher (8.40%) compared to the blend samples and pearl millet flour (control). As the proportion of pearl millet flour in the flour blends increases, the crude fat content of the flour blends significantly decreased (Table 1). The fat content of the fermented pearl millet-acha flour blends ranged from 5.31 to 6.27% (Table 1). Fermentation also decreased the fat content of the pearl millet-acha flour blends (Table 1). Fat content ranging from 2.7 to 7.1% has been reported for some pearl millet genotypes flours (Abdalla et al., 1998). Fat content values of 5.7, 5.6, 7.2 and 2.4% for raw, germinated, roasted and fermented millet flours, respectively, have been reported (Sade, 2009). Crude fat contents

ranging from 2.1 to 2.5% has been reported for acha (*D. exilis*) (Chukwu and Abdul-kadir, 2008; Irving and Jideani, 1997; Jideani, 1999). The reduction in fat contents of pearl millet-acha flour blends with increasing proportions of pearl millet flour could be due to the low fat composition of the pearl millet grains (Table 1). Decreased fat content has been reported for fermented millet flour (Sade, 2009).

The ash content of unfermented acha flour was 2.22 %, and that for unfermented pearl-millet flour blends ranged from 1.67 to 1.96% (Table 1). Increasing the proportion of pearl millet flour significantly (p ≤ 0.05) decreased the ash contents of the flour blends. However, no significant (p > 0.05) differences were observed between the ash content of unfermented 100 % pearl millet (E, control) and 5:1 pearl millet to acha flour blend (D) samples. Similarly, the ash content of 3:1 pearl millet to acha flour blend (B) and 4:1 pearl millet to acha flour blend (C) were not significantly different. The ash content of the fermented pearl millet-acha flour blends ranged from 3.68 – 5.00 % (Table 1). In contrast to the unfermented pearl millet-acha flour blends, the ash content in the

fermented pearl millet-acha flour blends significantly (p ≤ 0.05%) increased with increasing proportion of pearl millet flour. Fermentation significantly increased the ash content of the pearl millet-acha flour blends (Table 1). Abdalla et al. (1998) reported ash content values ranging from 1.8 to 2.4% for flours from pearl millet genotypes. Ash content of 2.1, 2.1, 2.7 and 1.9% for raw, germinated, roasted and fermented millet flours, respectively, have been reported (Sade, 2009). Ash content values of 1.4% (Irving and Jideani, 1997) and 3.6% (Chukwu and Abdul-kadir, 2008) have been reported for acha (*D. exilis*). The reduction in ash content of pearl millet-acha flour blends with increasing proportions of pearl millet flour could be due to the low ash content of the pearl millet grains (Table 1). Decreased ash content has been reported for fermented millet flour (Sade, 2009). The fibre content of unfermented pearl millet-acha flour blends ranged from 1.97 to 2.28% compared to 2.80 % for acha flour (Table 1). Increased proportion of pearl millet flour significantly (p ≤ 0.05) decreased the fibre content of the pearl millet- acha flour blends. However, the fibre content between 4:1 pearl

millet to acha flour blend (C) and 5:1 pearl millet to acha flour blend (D) were not significantly different. The fibre content of fermented pearl millet-acha flour blends ranged from 0.65 to 1.43% (Table 1). Fibre content of fermented pearl millet-acha flour blends significantly increased with increased proportion of pearl millet flour. However, no significant differences were observed between fermented flour blends from 100% pearl millet (E, control) and 5:1 pearl millet to acha flour blend (D). Fermentation significantly ($p ≤ 0.05$) decreased the fibre content of the pearl millet-acha flour blends (Table 1). Fibre content ranging from 2.6 to 4.0% have been reported for pearl millet genotypes flours (Abdalla et al., 1998). Fibre content of 2.0, 1.8, 1.8 and 1.8%, respectively, have been reported for flours from raw, germinated, roasted and fermented millet (Sade, 2009). Fibre content of 0.48% (Irving and Jideani, 1997) and 1.9% (Chukwu and Abdul-kadir, 2008) have been reported for acha (*D. exilis*). The decrease in fibre content of pearl millet-acha flour blends with increasing proportions of pearl millet flour could be due to the low fibre content of the pearl millet grains (Table 1). The reduction in fibre contens of fermented flour blends could be attributed to enzymatic breakdown of fibre during fermentation by the lactic acid bacteria (Ojokoh et al., 2013; Ojokoh et al., 2014). Decreased fibre content has been reported for fermented millet flour (Sade, 2009). Amankwah et al. (2009) reported increased crude fibre content in fermented maize flour.

The protein content of unfermented pearl millet-acha flour blends ranged from 8.00 to 11.52% compared to 20.15% for acha flour (Table 1). Increasing the proportions of the pearl millet flour significantly ($p ≤ 0.05$) decreased the protein content of the pearl millet-acha flour blends. The protein content of the fermented pearl millet-acha flour blends ranged from 9.52 to 18.52% (Table 1). As the proportion of pearl millet flour increased, the protein content of the fermented pearl millet-acha flour blends significantly ($p ≤ 0.05$) increased and then drop after 4:1 pearl millet to acha flour blend (C) sample. Fermentation significantly ($p ≤ 0.05$) increased the protein content of the pearl millet- acha flour blends. Protein content values ranging from 8.5 to 15.1% have been reported for pearl millet genotypes flours (Abdalla et al., 1998). Crude protein content values of 14.0, 19.4, 15.7 and 17.5% have been reported for flours from raw, germinated, roasted and fermented millet, respectively (Sade, 2009). Protein content values ranging from 6.9 to 10.6% have been reported for acha (*D. exilis*) (Chukwu and Abdul-kadir, 2008; Irving and Jideani, 1997; Jideani, 1999). The reduction in protein content of the blends with increasing proportions of pearl millet could be explained by the low content of protein in pearl millet (Table 1). The increases in crude protein content recorded with fermentation could be due to the activities and increase in number of microorganisms (Ojokoh et al., 2013; Ojokoh et al., 2014). Increase in protein content has been

reported in fermented maize flour (Amankwah et al., 2009) and fermented millet flour (Sade, 2009). Increase in protein content has been attributed to proteolytic activities of enzymes produced by microorganisms during fermentation (Amankwah et al., 2009).

Carbohydrate content of unfermented pearl millet-acha flour blends ranged from 70.99 to 76.82% compared to 59.22% for acha flour (Table 1). Increased proportions of pearl millet flour contributed to significant ($p ≤ 0.05$) increase in carbohydrate content of pearl millet-acha flour blends. However, no significant ($p > 0.05$) differences were observed between the carbohydrate contents of 4:1 pearl millet to acha flour blend (C) and 5:1 pearl millet to acha flour blend (D). Carbohydrate content of fermented pearl millet-acha flour blends ranged from 57.80 to 68.30% (Table 1). As the proportion of pearl millet flour increased, the carbohydrate content of the fermented pearl millet-acha flour blends significantly ($p ≤ 0.05$) decreased and then increased after 5:1 pearl millet to acha flour blend (D) sample. Fermentation significantly ($p ≤ 0.05$) decreased the carbohydrate content of the pearl millet-acha flour blends. Carbohydrate content of 76.3, 71.1, 72.6 and 76.5% have been reported for flours from raw, germinated, roasted and fermented millet, respectively (Sade, 2009). Carbohydrate content ranging from 81.8 to 87.8% has been reported for acha (*D. exilis) (*Irving and Jideani, 1997; Jideani, 1999). The increase in carbohydrate content with increasing pearl millet proportion could be due to high carbohydrate composition of the pearl millet (Table 1). The reduction in carbohydrate content with fermentation could be attributed to utilization of fermentable sugars by lactic acid bacteria for growth and other metabolic activities (Ojokoh et al., 2013).

Physico-chemical properties

pH and titratable acidity

The changes in pH and titratable acidity of pearl millet-acha flour blends during fermentation are represented in Figure 1. pH values for all the samples before fermentation (time 0) ranged from 4.55 to 4.82. The pH values for pearl millet-acha flour blends before fermentation were significantly ($p ≤ 0.05$) different from each other. During the fermentation period, pH values decreased significantly for all the samples (Figure 2A). However, pH values for 4:1 pearl millet-acha flour blend fermenting medium (C) and 5:1 pearl millet-acha flour blend fermenting medium (D) at 48 h were not significantly different from each other. Similarly, pH values for 2:1 pearl millet-acha flour blend fermenting medium (A) and 100% pearl millet flour blend fermenting medium (E) at 72 h were not significantly different from each other.

Decreases in pH of the fermenting pearl millet-acha flour
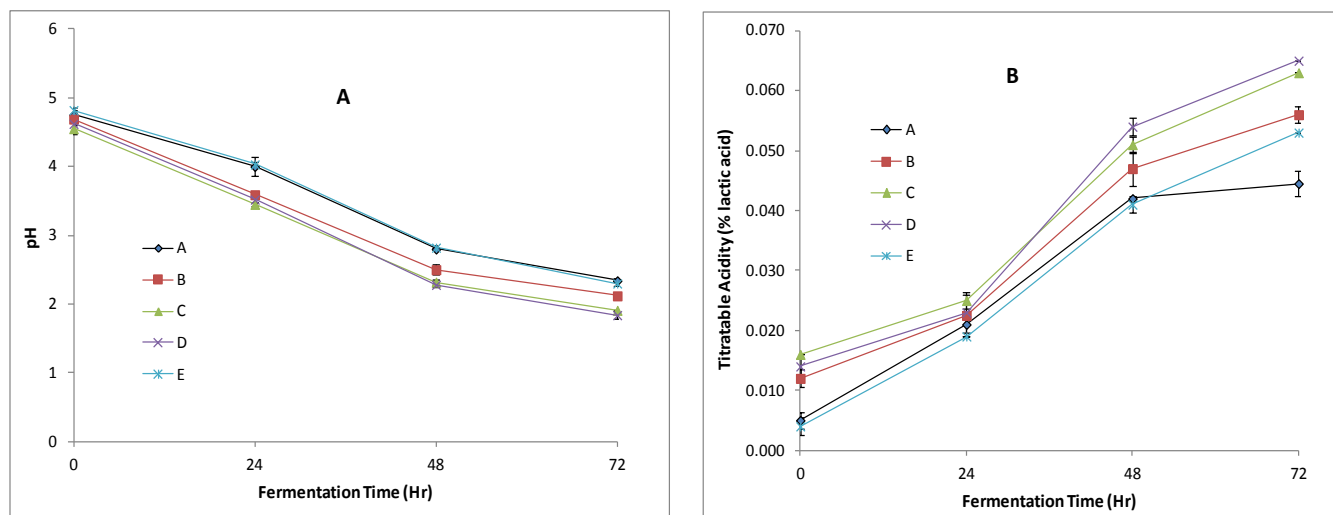
Figure 2. Changes in pH (**A**) and titratable acidity (**B**) of sprout pearl millet-acha flour blends during fermentation period. Error bars = ± standard deviations. A = 2:1 Pearl millet to acha blend, B = 3:1 Pearl millet to acha blend, C = 4:1Pearl millet to acha blend, D = 5:1 Pearl millet to acha blend, E = 100 % Pearl millet (Control).

Table 2. Morphological and biochemical characteristics of lactic acid bacteria isolated during the spontaneous fermentation of pearl millet-acha flour blends.

Cellular morphology	Gram reaction	Catalase	Oxidase	Motility test	Growth at 4°C	Growth at 45°C	Growth at pH 3.9	NH3 from Arginine	H2S production	Growth at 4% Nacl	Homo/Hetero-fermentation	Methyl Red	Voges Proskaeur	Glucose	Xylose	Raffinose	Sucrose	Fructose	Mannose	Maltose	Mannitol	Trehalose	Arabinose	Rhamnose	Lactose	Galactose	Probable organism
Rods	+	-	-	-	-	+	+	-	-	-	HM	+	-	+	-	-	+	+	-	-	-	+	-	+	+	+	*Lactobacillus acidophilus*
Rods	+	-	-	-	+	-	+	-	+	+	HM	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Rods	+	-	-	-	+	-	+	+	-	-	HE	+	-	+	+	+	+	-	-	+	+	-	+	-	+	+	*Lactobacillus brevis*
Rods	+	-	-	-	+	+	+	+	-	+	HM	+	-	+	-	-	+	+	-	+	-	-	-	-	+	+	*Lactobacillus leichmannii*
Rods	+	-	-	-	-	-	+	+	-	-	HE	+	-	+	-	+	+	-	+	+	-	+	+	-	+	+	*Lactobacillus cellobiosus*
Cocci	+	-	-	-	+	+	+	-	-	+	HM	+	-	+	+	-	+	-	-	-	-	-	-	-	+	+	*Pediococcus* sp
Rods	+	-	-	-	+	-	+	-	-	+	HM	+	-	+	-	-	+	+	+	+	+	-	+	+	+	+	*Lactobacillus casei*
Cocci	+	-	-	-	+	+	+	-	-	+	HE	+	-	+	-	-	+	+	+	+	-	-	+	-	+	+	*Streptococcus thermophillus*

+ = positive test; - = negative test; HE = Heterofermentative HM = Homofermentative.

Table 3. Morphological and biochemical characteristics of bacterial isolates from raw and during fermentation of pearl millet–acha flour blends.

Morphological Characteristics	Gram reaction	Spore test	Motility	Catalase	Oxidase	Coagulase	Urease	Glucose	Sucrose	Mannitol	Lactose	Starch hydrolysis	Probable bacteria
Creamy, punctiforms, Umbonate, entire and wrinkled cocci	+	-	-	+	+	-	+	NAG	NAG	NAG	NAG	-	*Micrococcus luteus*
Creamy white, irregular, Raised, undulate and rough surface	+	+	+	+	+	-	-	AG	NAG	AG	NAG	+	*Bacillus subtilis*
Milky, irregular, Raised, entire, opaque and wet surface	-	-	+	+	+	-	-	NAG	NAG	NAG	NAG	-	*Pseudomonas aeruginosa*
Circular, convex, creamy, entire and smooth surface	+	-	-	+	-	+	-	ANG	ANG	AG	ANG	-	*Staphylococcus aureus*
Cream, irregular, raised, entire and rough surface	+	-	+	+	+	-	+	AG	AG	NAG	NAG	-	*Proteus vulgaris*

+ = positive test; - = negative test; AG = Acid and Gas production; NAG = No Acid and Gas production; ANG = Acid production and no Gas.

blend medium were accompanied by significant ($p \leq 0.05$) increases in titratable acidity (Figure 2B). The decrease in pH with simultaneous increase in titratable acidity during fermentation has been reported for millet flour (Khetarpaul and Chauhan, 1989), millet-acha based *kunun zaki* (beverage) (Ayo, 2004), breadfruit-cowpea blend flours (Ojokoh et al., 2013, 2014) and maize flour (Amankwah et al., 2009). The reduction in pH and simultaneous increase in TTA during fermentation could be attributed to the activities of lactic acid bacteria (Ojokoh et al., 2013). Lactic acid bacteria have been reported to degrade carbohydrates (Table 1) resulting in acidification (Ojokoh et al., 2013).

Microbial characteristics

A total of 13 bacteria were identified both in raw and during fermentation of the blend samples (Tables 2 and 3). *Micrococcus luteus, Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from the raw sample and at the early stage of the fermentation of the blends followed by disappearance towards the end of fermentation process. Studies have reported the presence of *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* in similar products and were attributed to inadequate precautionary measures during processing (Adeyemi and Umar 1994; Osuntogun and Aboaba, 2004). However, the disappearance of these organisms as the fermentation progressed had been reported in a similar product known as 'koozh' an Indian fermented millet beverage (Ilango and Antony, 2014). Several studies had shown that fermentation of cereals helps in altering the pH to levels that do not favour growth of pathogenic bacteria due to the production of antimicrobial compounds by the LAB during fermentation. (Steinkraus, 2002; Hernández-Ledesma et al., 2004; Adams and Nicolaides, 1997; Odumodu and Inyang, 2006; Oliveira et al., 2014). Lactic acid bacteria (LAB) were the prevalent bacteria during the fermentation of pearl millet and acha flour blends with the *L. plantarum* been the most dominant LAB. This is similar to the findings of Adeyemi and Umar (1994) who reported LAB to be the most prevalent bacteria associated with the fermentation and production of "kunu-zaki" made from sorghum and millet blends.

In conclusion, the findings from this study suggest that fermentation of pearl millet-acha flour blends with the purpose of preparing traditional beverages can improve their nutritional composition.

Conflict of Interest

The authors have not declared any conflict of interest.

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