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Nutritional and Antioxidant Properties of Fermented Kunu Using *Lactobacillus plantarum, Lb. fermentum, Bacillus subtilis* and *Saccharomyces cerevisiae* Isolates

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ABSTRACT

Recently, African researchers have been looking for ways to improve the nutritional components of some fermented indigenous foods using natural materials that are environmentally friendly. This study aimed to investigate the nutritional and antioxidant properties of fermented kunu using *Lactobacillus plantarum, Lb. fermentum, Bacillus subtilis* and *Saccharomyces cerevisiae* isolates. Isolation of microorganisms from the kunu samples using serial dilution and pour plate techniques was conducted. Fermentation of kunu using *Lb. plantarum, Lb. fermentum, B. subtilis* and *S. cerevisiae* isolates was evaluated using standard inoculation methods. The nutritional and antioxidant properties of the kunu were then determined. The microorganisms isolated from the kunu samples included *Serratia marcescens, Staphylococcus aureus, Bacillus subtilis, Lactobacillus plantarum, Lb. fermentum, Aspergillus niger, A. flavus, Escherichia coli* and *Saccharomyces cerevisiae*. The inoculants used were *Lb. plantarum, Lb. fermentum, S. cerevisiae* and *B. subtilis*. The protein (4.01%), ash (2.54%), fibre (6.98%) and moisture contents (85.01%) of kunu from mono- and co-cultural fermentation increased significantly, while fat (1.14%) and carbohydrate (6.70%) decreased when compared with naturally fermented kunu. The total phenolic (3.34 mg/g) and flavonoid (1.40 mg/g) contents of kunu from mono- and co-cultural fermentation increased compared with naturally fermented kunu. Therefore, the improvement in the nutritional content of the fermented kunu sample with synergistic action of the microorganisms suggests that it can be a good source of protein and probiotics.

Keywords: Nutrition, Antioxidant, Protein, Fermentation, Inoculation.

INTRODUCTION

Cereals such as millet, maize (*Zea mays*), sorghum (*Sorghum bicolor*) and barley (*Hordeum vulgare*) form major food components in various traditional diets, especially among the less affluent individuals in society (Amit & Yashbir, 2024). Consumption of millet and millet products confers considerable therapeutic and nutritional benefits (Gahalawat *et al*., 2024).

Local fermentation technology is employed in the processing of millet into desirable products with high nutritional contents (Ajagekar *et al*., 2023). Fermentation is the main precursor in the production of different fermented products, enhancing the nutritional composition of foods through the biosynthesis of vitamins, essential amino acids and proteins, improved bioavailability of micronutrients, and the degradation of antinutritional factors (Senanayake *et al*., 2023). It has been reported that fermentation promotes food safety, reduces food toxicity and aids in the production of antimicrobial agents (Sharma *et al*., 2020).

Millet can be processed into various forms such as ogi, fura, burukutu, bushera, koko, mangisi, jandh, togwa, ben-saalga, uji, dambu, roti, and kunu. Kunu is a non-alcoholic fermented beverage produced from millet. It is widely consumed in the northern parts of Nigeria, especially during the dry season (Olatoye *et al*., 2023). Kunu forms an essential component of the diet in many developing countries and is consumed either as a main dish or as a condiment (Ndukwe *et al*., 2023).

Kunu processing is commonly carried out by women using simple household equipment and utensils. The processing of kunu can be fortified using different spices such as ginger, clove, black pepper and ehuru (Adeniji & Ayoade, 20214). The microorganisms involved during kunu processing play active roles in the physical, nutritional and organoleptic modification of the substrates (Ndukwe *et al*., 2023). Mono- and co-cultural techniques have proven successful in enhancing the nutritional components of most fermented foods and in the synthesis of biomolecules (Omotade *et al*., 2024). Recently, little information has been available on the mono- and co-cultural potential of the microorganisms isolated from kunu.

Although fermentation of millet for kunu production using traditional processing methods involving natural microflora has proven successful in terms of nutritional quality, this study was designed to evaluate the nutritional and antioxidant composition of fermented kunu using different microorganisms isolated from kunu.

MATERIAL AND METHODS

Collection of Samples

Five different kunu samples (500 ml each) were purchased from different hawkers in various locations in Akure metropolis, namely Nepa Market, Oja-Oba Market, Isinkan Market, Ilesha Garage (Motor Park) and the Federal University of Technology, Akure Junior Staff Quarters. The samples were collected in sterile bottles. The raw millet (*Pennisetum glaucum*) (500 g) was also purchased at Isinkan Market, Akure, in sterile transparent polyethylene bags, well labelled, and immediately transported to the Microbiology Laboratory, Federal University of Technology, Akure for further analyses.

Preparation of the Kunu

This was carried out according to the method described by Abidoye (2017). Approximately 500 g of cleaned millet were weighed, steeped, washed and wet milled with the addition of spices (ginger, red pepper, black pepper and cloves). The wet-milled millet was sieved, and the supernatant was decanted to obtain a clear slurry. The slurry was divided into two unequal parts; two-thirds (75%) was added to boiling water, stirred and then cooled to 35 ± 2 °C; subsequently, the remaining one-quarter (25%) was added. The mixture was thoroughly stirred and sweetened with 10% granulated sugar. The product obtained (kunu) was divided into two portions; one portion was packed into sterile plastic bottles and pasteurised at 75 °C for 30 minutes and then allowed to cool, while the other portion (unpasteurised) served as the control.

Microbiological Analysis

Ten millilitres (10 ml) of each sample were homogenised in 90 ml of sterile peptone water to form the stock cultures. One millilitre (1.0 ml) from the stock culture was pipetted and serially diluted to appropriate dilutions of 10^{-5} and 10^{-3} for bacteria and fungi, respectively. Nutrient agar, de Man's Rogosa Sharpe agar (MRSA), potato dextrose agar and yeast extract agar were prepared according to the manufacturers' guidelines and used for culturing total bacterial, lactic acid bacterial, fungal and yeast isolates, respectively. Fungal plates were incubated at 28 °C for 72–96 hours, bacterial plates were incubated at 37 °C for 24 hours, and MRS agar plates were incubated anaerobically at 28 °C for 24–48 hours. Pure isolates were obtained by repeated streaking on freshly prepared microbiological media. Characterisation and identification of the isolated microorganisms were based on

their cultural, microscopic, morphological and biochemical tests (Muthukumar & Dakshayini, 2021).

Inoculum Preparation

The inocula were prepared using 0.5 McFarland turbidity standards. The McFarland solution was prepared by adding 9.95 ml of 1% H_2SO_4 to 0.05 ml of 1% $BaCl_2$. The absorbance of the solution was then checked using a spectrophotometer at 623 nm. The microbial suspensions were prepared by adding a fresh culture of the isolates into sterile distilled water in a test tube until the turbidity matched the McFarland standard, representing 1.5×10^8 cfu/ml for bacteria and 1.5×10^8 sfu/ml for yeast suspension. Two hundred millilitres (200 ml) of pasteurised kunu was distributed into different fermenting vessels. Bacterial suspensions of 1.0×10^3 cfu/ml and 1.0×10^3 sfu/ml of yeast suspension each were used for mono- and co-cultural fermentation at ambient temperature (Eduardo *et al.*, 2018).

Total Titratable Acidity and pH

The pH and titratable acidity of the kunu were monitored over 2 days. The percentage total titratable acidity was determined by diluting 10 ml of the sample in 90 ml of sterile distilled water. The mixture was homogenised and allowed to settle, from which 20 ml was titrated against 0.1 M NaOH using phenolphthalein as an indicator (Ire *et al.*, 2020). The pH was determined using a pH meter (Crison Basic model 20) calibrated with standard buffers (pH 7.0 and 4.0).

Proximate Composition

The proximate composition of the fermenting kunu was determined according to AOAC (2012). The parameters measured included moisture, crude protein, crude fat, ash, fibre and carbohydrate contents.

Antioxidant Factors

The antioxidant properties of the kunu were determined as described by Zhishen *et al.* (1999), modified by Olatoye *et al.* (2023). The antioxidant factors measured were total phenolic and flavonoid contents.

Statistical Analysis

The data obtained were analysed and are presented as mean ± standard deviation. The significance of differences between groups was tested using one-way analysis of variance (ANOVA) with SPSS Windows Version 23 software. Differences were considered statistically significant at $p < .05$.

RESULTS

Total Microbial Counts from Kunu Samples

Table 1 shows the total microbial counts from kunu samples. The sample purchased from Nepa Market, Akure had the highest bacterial count (20.33 × 10^5 cfu/ml on nutrient agar), followed by Isinkan Market, Akure (19.00 × 10^5 cfu/ml), while the lowest bacterial count (3.00 × 10^5 cfu/ml) was obtained from the prepared sample on MRS agar. Similarly, the highest fungal count (7.67 × 10^3 sfu/ml) was obtained from the sample purchased from Ilesha Garage, Akure on yeast extract agar, followed by the sample from Nepa Market, Akure on potato dextrose agar, while the lowest fungal count (2.33 × 10^3 sfu/ml) was obtained from the prepared sample on yeast extract agar.

Table 1: Total Microbial Counts Obtained from Kunu Samples

Sample source	NA (cfu/ml x10^5)	MRS (cfu/ml x10^5)	YEA (sfu/ml x10^3)	PDA (sfu/ml x10^3)
JSQ	7.00±0.58 ^{b}	9.33±0.33^{b}	2.33±0.33^{bc}	3.67±0.33^{a}
IMA	19.00±0.58^{e}	12.33±0.88^{cd}	3.67±0.67^{b}	3.67±0.67^{a}
OMA	10.00±0.00^{c}	11.00±0.58^{bc}	3.67±0.33^{b}	4.67±0.33^{a}
NMA	20.33±0.33^{f}	9.67±0.88^{b}	1.67±0.33^{a}	7.33±0.67^{b}
IGA	12.33±0.58^{d}	14.00±0.58^{d}	7.67±0.67^{c}	3.67±0.88^{a}
PKL	5.00±0.00^{a}	3.00±0.58^{a}	2.33±0.33^{bc}	3.33±0.33^{a}

Data are represented as mean ± standard deviation. Means in the same column with different superscripts are significantly different ($p < .05$). Key: NA = Nutrient agar; MRS = de Man's Rogosa Sharpe agar; YEA = Yeast extract agar; PDA = Potato dextrose agar; JSQ = Junior Staff Quarters, FUTA; IMA = Isinkan Market, Akure; OMA = Oja-Oba Market, Akure; NMA = Nepa Market, Akure; IGA = Ilesha Garage, Akure; PKL = Prepared kunu in laboratory.

Colonial, Morphological and Biochemical Characterisation of Yeast and Bacterial Isolates

The colonial, morphological and biochemical characterisation of yeast and bacterial isolates from kunu samples is presented in Table 2.

Table 2: Colonial, Morphological and Biochemical Characterisation of Yeast and Bacterial Isolates from Kunu Samples

Isolate codes	Colony morphology	Gram reaction	Motility	Catalasee	Oxidase	Methyl red	Coagulase	Spore forming	Indole test	Urease test	Starch Hydrolysis	Ornithine	Lactose	Sucrose	Galactose	Maltose	Mannitol	Glucose	Probable identity
X1	CRO	-SR	+	+	-	+	ND	-	+	-	ND	ND	A	A	A	A	A	A	*Escherichia coli*
X2	RIR	-R	+	+	-	-	ND	-	-	-	ND	ND	A	A	A	A	A	A	*Serratia marcescens*
X3	CSY	+C	-	+	-	+	+	-	-	+	ND	ND	AG	A	A	A	A	A G	*Staphylococcus aureus*
X4	LRC	+R	+	-	-	+	ND		-	-	+	-	A	A	A	AG	A	A G	*Lactobacillus plantarum*
X5	GWI	+LR	+	+	-	-	ND	+	-	-	+	ND	AG	A	A	A	AG	A	*Bacillus subtilis*
X6	SRC	+SR	+	-	-	-	ND	-	-	-	+	+	AG	AG	AG	AG	AG	A G	*Lb. fermentum*
X7	IB	+	ND	+	ND	ND	ND	ND	ND	+	ND	ND	-	A	ND	ND	ND	A	*S. cerevisiae*

Keys: CRO = cream, opaque, round and flat elevation; RIR = red irregular, raised; CSY = circular, small, yellow; LRC = long rod, creamy; GWI = grey white, opaque irregular shape; SRC = short rod; R = rod; C = cocci; LR = long rod; ND = not determined; (–) = negative; (+) = positive; AG = acid production with gas; A = acid production; IB = internal budding.

Cultural Morphology and Microscopic Observation of Mold Isolated from Kunu Samples

The cultural morphology and microscopic observation of fungal isolates from kunu samples are presented in Table 3. The probable identities of the fungal isolates were *Aspergillus flavus* and *A. niger*

Table 3: Cultural Characterisation and Microscopic Observation of Mold Isolated from Kunu Samples

Isolates codes	Cultural characteristics	Microscopic observation	Probable identity
PDA1	Fast growing organisms, black to yellow colour in appearance	Septate mycelium with long hyphae, long conidiosphores with spherical vesicle at the apex, conidia are globose vesicle, phalliades, regularly roughened and uninucleated	*Aspergillus flavus*
PDA2	Typically black powdering, with dark spores varying in sizes	Septate mycelium with long hyphae, long conidiosphores with spherical vesicle at the apex, conidia are globose vesicle, phalliades, regularly roughened and uninucleate	*A. niger*

Key: PDA = Potato dextrose agar

pH of Kunu from Mono- and Co-Cultural Fermentation

The pH of kunu from mono- and co-cultural fermentation is represented in Table 4. The highest pH value (7.40) was obtained in the sample inoculated with *B. subtilis,* while the lowest pH (3.40) was obtained with the kunu sample inoculated with *Lb. fermentum* and *Lb. plantarum* at 48 hours of fermentation.

Table 4: pH of Kunu from Mono- and Co-cultural Fermentation

ISOLATES	0 HOUR	24HOURS	48 HOURS
SAC+LPT	6.40±0.00^{a}	4.00±0.02^{a}	3.91±0.04^{b}
SAC+LFT	6.40±0.00^{a}	4.01±0.02^{a}	3.80±0.15^{b}
SAC+BSU	6.41±0.01^{a}	5.58±0.01^{c}	5.36±0.01^{e}
LPT+BSU	6.40±0.12^{a}	4.96±0.01^{b}	4.56±0.01^{c}
LFT+BSU	6.39±0.03^{a}	4.95±0.03^{b}	4.60±0.08^{c}
LPT+LFT	6.39±0.18^{a}	3.89±0.33^{a}	3.40±0.13^{a}
SAC	6.40±0.03^{a}	5.85±0.03^{d}	5.21±0.01^{d}
LPT	6.40±0.00^{a}	4.00±0.15^{a}	3.85±0.03^{b}
LFT	6.40±0.03^{a}	4.01±0.01^{a}	3.82±0.13^{b}
BSU	6.40±0.00^{a}	6.83±0.01^{e}	7.40±0.18^{f}
NFM	6.40±0.00^{a}	6.00±0.05^{de}	5.84±0.15e

Values are means of triplicate determinations ± standard deviation. Means in the same column with different superscripts are significantly different ($p < .05$). Key: SAC = Saccharomyces cerevisiae; LPT = Lactobacillus plantarum; LFT = Lb. fermentum; BSU = Bacillus subtilis; SAC+LFT = S. cerevisiae and Lb. fermentum; SAC+LPT = S. cerevisiae and Lb. plantarum; SAC+BSU = S. cerevisiae and B. subtilis; LPT+LFT = Lb. fermentum and Lb. plantarum; LPT+BSU = Lb. plantarum and B. subtilis; LFT+BSU = Lb. fermentum and B. subtilis; NFM = Naturally fermented kunu.

Total Titratable Acidity of Kunu from Mono- and Co-cultural Fermentation

The total titratable acidity of kunu from mono- and co-cultural fermentation is represented in Table 5. The sample inoculated with *Lb. plantarum* and *Lb. fermentum* had the highest total titratable acidity (0.81) at 48 hours, while the sample inoculated with *B. subtilis* had the lowest value (0.13).

Table 5: Total Titratable Acidity of Kunu from Mono- and Co-cultural Fermentation

ISOLATES	0 HOUR	24 HOURS	48 HOURS
SAC+LPT	0.17±0.00^{a}	0.45±0.00^{d}	0.55±0.03^{d}
SAC+LFT	0.17±0.03^{a}	0.45±0.13^{d}	0.54±0.06^{d}
SAC+BSU	0.17±0.03^{a}	0.18±0.02^{a}	0.22±0.04^{b}
LPT+LFT	0.41±0.02^{b}	0.72±0.05^{e}	0.80±0.03^{f}
LPT+BSU	0.17±0.04^{a}	0.30±0.03^{c}	0.44±0.00^{c}
LFT+BSU	0.17±0.00^{a}	0.31±0.00^{c}	0.42±0.00^{c}
SAC	0.17±0.00^{a}	0.22±0.08^{b}	0.24±0.01^{b}
LPT	0.17±0.03^{a}	0.47±0.02^{d}	0.66±0.03^{e}
LFT	0.17±0.00^{a}	0.46±0.01^{d}	0.65±0.05^{e}
BSU	0.17±0.06^{a}	0.16±0.00^{a}	0.13±0.03^{a}
NFM	0.17±0.04^{a}	0.19±0.03^{a}	0.23±0.04^{b}

Values are means of triplicate determinations ± standard deviation. Means in the same column with different superscripts are significantly different ($p < .05$). Key: SAC = S. cerevisiae; LPT = Lb. plantarum; LFT = Lb. fermentum; BSU = B. subtilis; NFM = Naturally fermented kunu.

Proximate Composition of Kunu from Mono-Cultural Fermentation

The proximate composition of kunu from mono-cultural fermentation shows an increase in protein, ash, fibre and moisture content and a decrease in crude fat and carbohydrate content compared with naturally fermented kunu (Tables 6). Protein, ash, fibre and moisture contents increased from 1.37% to 2.98%, 0.46% to 1.70%, 6.32% to 6.98% and 84.01% to 85.01%, respectively, while crude fat and carbohydrate content decreased from 1.14% to 0.70% and 6.90% to 4.60%, respectively.

Table 6: Proximate Composition of Kunu from Mono-cultural Fermentation

Isolate	Protein	Fat	Ash	Carbohydrate	Fibre	Moisture contents
SAC	2.98±0.00^{e}	0.70±0.00^{a}	1.70±0.01^{c}	4.60±0.10^{a}	5.01±0.33^{a}	85.01±0.01^{d}
LPT	2.96±0.00^{d}	0.88±0.00^{b}	1.83±0.00^{d}	5.02±0.01^{b}	6.98±0.33^{d}	82.33±0.18^{a}
LFT	2.72±0.01^{c}	0.88±0.01^{b}	1.72±0.01^{c}	5.00±0.00^{b}	6.35±0.00^{c}	83.33±0.33^{b}
BSU	2.56±0.00^{b}	1.10±0.00^{c}	1.49±0.01^{b}	6.77±0.03^{c}	5.78±0.33^{b}	82.30±0.06^{a}
NFM	1.37±0.00^{a}	1.14±0.00^{d}	0.46±0.00^{a}	6.90±0.00^{c}	6.32±0.00^{c}	84.01±0.00^{c}

Values are means of triplicate determinations ± standard deviation. Means in the same column with different superscripts are significantly different ($p < .05$). Key: SAC = S. cerevisiae; LPT = Lb. plantarum; LFT = Lb. fermentum; BSU = B. subtilis; NFM = Naturally fermented kunu.

Proximate Composition of Kunu from Co-cultural Fermentation

The proximate composition of kunu from co-cultural fermentation shows an increase in protein and ash content and a decrease in crude fat, fibre, moisture content and carbohydrate content compared with naturally fermented kunu (Tables 7). Protein and ash content increased from 1.37% to 4.00% and 0.46% to 2.50%, respectively, while crude fat, fibre, moisture content and carbohydrate content decreased from 1.14% to 0.62%, 6.32% to 4.34%, 84.01% to 72.81% and 6.70% to 3.99%, respectively.

Table 7: Proximate Composition of Kunu from Co-cultural Fermentation

Isolate	Protein	Fat	Ash	Carbohydrate	Fibre	Moisture content
SAC+LFT	4.00 ± 0.01^{f}	0.63 ± 0.00^{b}	2.50 ± 0.00^{e}	4.04 ± 0.01^{b}	5.21 ± 0.18^{b}	72.81 ± 0.01^{a}
SAC+LPT	4.01 ± 0.00^{f}	0.62 ± 0.00^{a}	2.54 ± 0.01^{f}	3.99 ± 0.01^{a}	5.83 ± 0.01^{d}	80.01 ± 0.01^{f}
SAC+BSU	3.15 ± 0.00^{d}	0.89 ± 0.01^{f}	2.10 ± 0.01^{c}	4.36 ± 0.03^{d}	5.47 ± 0.33^{c}	79.03 ± 0.03^{e}
LPT+LFT	3.97 ± 0.00^{e}	0.69 ± 0.02^{c}	2.45 ± 0.01^{d}	4.23 ± 0.01^{c}	$5.64\pm0.34^{c,d}$	77.02 ± 0.01^{c}
LPT+BSU	2.98 ± 0.01^{b}	0.70 ± 0.00^{d}	2.00 ± 0.00^{b}	4.98 ± 0.01^{f}	4.34 ± 0.01^{a}	75.00 ± 0.00^{b}
LFT+BSU	3.06 ± 0.01^{c}	0.82 ± 0.00^{e}	2.00 ± 0.01^{b}	4.88 ± 0.00^{e}	6.24 ± 0.33^{e}	78.00 ± 0.00^{d}
NFM	1.37 ± 0.00^{a}	1.14 ± 0.00^{g}	0.46 ± 0.00^{a}	6.70 ± 0.00^{g}	$6.32\pm.00^{e}$	84.01 ± 0.00^{g}

Values are means of triplicate determinations ± standard deviation. Means in the same column with different superscripts are significantly different ($p < .05$). Key: SAC+LFT = S. cerevisiae + Lb. fermentum; SAC+LPT = S. cerevisiae + Lb. plantarum; SAC+BSU = S. cerevisiae + B. subtilis; LPT+LFT = Lb. fermentum + Lb. plantarum; LPT+BSU = Lb. plantarum + B. subtilis; LFT+BSU = Lb. fermentum + B. subtilis; NFM = Naturally fermented kunu.

Antioxidant Profile of Kunu from Mono- And Co-Cultural Fermentation

The antioxidant profile of kunu from mono- and co-cultural fermentation is presented in Table 8. The results showed a considerable increase in the flavonoid and phenolic content of kunu from mono- and co-cultural fermentation compared with naturally fermented kunu. The highest flavonoid content of 1.40 mg/g and phenolic content of 3.34 mg/g were observed in the sample fermented with *Lb. plantarum* and *Lb. fermentum*, compared with naturally fermented kunu.

Table 8: Antioxidant Profile of Kunu from Mono- and Co-cultural Fermentation

Isolates	Flavonoid content (mg/g)	Phenolic content (mg/g)
S. cerevisiae and *L. fermentum*	1.37±0.02^{f}	3.23±0.03^{ef}
S. cerevisiae and *L. plantarum*	1.38±0.01^{f}	3.15±0.01^{e}
S. cerevisiae and *B. subtilis*	1.23±0.05^{d}	3.00±0.03^{d}
L. plantarum and *L. fermentum*	1.40±0.00^{g}	3.34±0.06^{f}
L. plantarum and *B. subtilis*	1.13±0.00^{b}	3.02±0.04^{d}
B. subtilis and *L. fermentum*	1.30±0.02^{e}	2.99±0.01^{cd}
S. cerevisiae	1.18±0.03^{c}	2.86±0.03^{c}
L. plantarum	1.23±0.01^{d}	3.02±0.01^{d}
L. fermentum	1.20±0.02^{cd}	3.00±0.00^{d}
B. subtilis	1.00±0.08^{a}	2.12±0.03^{b}
Naturally fermented *kunu*	1.11±0.00^{b}	2.04±0.01^{a}

Values are means of triplicate determinations ± standard deviation. Means in the same column with different superscripts are significantly different ($p < .05$).

DISCUSSION

Adequate utilisation of fermented foods depends on the availability of nutrients that meet consumer needs. Many fermented foods are well known but remain underutilised due to the presence of antinutritional factors and improper processing. Fermentation techniques contribute to improved nutritional profiles and reduced antinutritional contents of fermented foods (Anyiam *et al.*, 2023). Various microorganisms associated with fermented foods significantly enhance their organoleptic properties. Several researchers have reported the isolation of diverse microorganisms from fermented cereal-based local beverages (Bouakkaz *et al.*, 2024). The highest microbial counts obtained from Nepa Market, Akure, may be due to the processing methods used, contamination from handlers, processing utensils and exposure to air or dust at the point of sale (Erinle & Ajayi, 2022). Conversely, lower microbial counts may be attributed to better hygienic conditions and controlled processing environments. For example, the highest bacterial counts

(8.00 cfu/ml) from kunu drinks sold in Port Harcourt metropolis have been reported (Essien *et al.*, 2009). However, it is generally stated that the standard limit of $<10^5$ cfu/ml is permissible for aerobic mesophilic bacteria in food (FDA, 2013).

The occurrence of *Staphylococcus aureus, Serratia marcescens, E. coli, Aspergillus niger* and *A. flavus* may be attributed to opportunistic contamination from air, human activity, or the natural flora of the grains used, as well as contamination from equipment (e.g. milling machines) employed in the preparation of kunu. The use of *Lb. fermentum, Lb. plantarum, S. cerevisiae* and *B. subtilis* in the fermentation process of kunu samples was based on reports that these microorganisms improve the nutritive value of some fermented foods (Bouakkaz *et al.*, 2024). Lactic acid bacteria, notably *Lactobacillus plantarum* and *Lb. fermentum*, and yeast, which are typically present in fermented drinks or foods, serve as biopreservatives, thereby enhancing the organoleptic and nutritional properties of fermented foods (Wang *et al.*, 2021). Zhina *et al.* (2021) suggested that yeast and bacteria exist in a symbiotic association, with the bacteria providing the acidic environment required for yeast growth, while the yeast supplies vitamins and other growth factors for the lactic acid bacteria.

The results of this study showed a decrease in pH and an increase in total titratable acidity of kunu from mono- and co-cultural fermentation compared with naturally fermented kunu. This increase in acidity and decrease in pH may be attributed to the abundant production of organic acids, such as lactic acid, by these microorganisms (Sionek *et al.*, 2023). These findings agree with those of Nwachukwu *et al.* (2010), who reported a decrease in pH of akamu and kunu during fermentation using lactic acid bacteria.

The proximate composition of kunu from mono- and co-cultural fermentation revealed an increase in ash, fibre, crude protein and moisture content, and a decrease in carbohydrate and fat content compared with naturally fermented kunu. The increase in crude protein content in inoculated kunu may be due to the high number of microorganisms capable of secreting extracellular proteolytic enzymes into the fermenting medium (Jabbar *et al.*, 2021). Adedire *et al.* (2017) reported an increase in protein content from 4.41% to 5.18% with enhanced fermentation of kunu by lactic acid bacteria.

The ash content of a sample is a rough measure of its inorganic mineral elements. The proximate analysis revealed an increase in the ash content of kunu from mono- and co-cultural fermentation compared with naturally fermented samples. This

increase may be due to the partial consumption of minerals by fermenting microorganisms during metabolism (Kiczorowski *et al.*, 2022), corroborating the findings of Sanni and Adesulu (2013) who also observed an increase in ash content during the fermentation of maize for masa production using starter cultures.

The crude fibre content of kunu from mono- and co-cultural fermentation increased relative to naturally fermented kunu. This increase could be attributed to the fermenting microorganisms preferentially utilising carbohydrates and fats in the medium as energy sources, thereby leaving a higher proportion of fibre (Sumaira *et al.*, 2022).

The total lipid content of kunu from mono- and co-cultural fermentation decreased compared with naturally fermented kunu. This reduction might be due to the utilisation of lipids by microorganisms as an energy source, sparing proteins from being used for energy (Olsen *et al.*, 2021). These results align with those of Ndulaka *et al.* (2014), who observed a decrease in fat content during the production and evaluation of reconstituted kunu, although they contrast with the findings of Adedire *et al.* (2017), who reported an increase in fat content during enhanced fermentation of kunu.

The carbohydrate content of kunu from mono- and co-cultural fermentation decreased relative to naturally fermented kunu, possibly due to the amylolytic enzymes produced by the inoculum or the utilisation of carbohydrates as an energy source by the fermenting microorganisms, which is consistent with previous studies (Xavier & Raj, 2011; Ndukwe *et al.*, 2023; Osman, 2011; Saleh *et al.*, 2013).

Moisture content significantly influences the microbial flora of fermented beverages. The increase in moisture content observed in kunu from mono- and co-cultural fermentation compared with naturally fermented kunu may be due to the availability of free water, autolysis of microorganisms and the inherent high water content of the medium. Adedeji and Oluwalana (2014) reported a similar increase in moisture content in the development and quality evaluation of a non-alcoholic beverage, which concurs with the findings of Agarry *et al.* (2010) during the production of kunu using starter cultures.

The flavonoid and phenolic contents of kunu from mono- and co-cultural fermentation increased compared with naturally fermented kunu. This increase may result from enhanced metabolic activities of the fermenting microorganisms, and enzymes such as β-glycosidase, which hydrolyse complex phenolic compounds

into simpler forms, thereby increasing the total phenolic content (Mousavi *et al.*, 2011). Other enzymes, such as proteases, may also modify the beverage composition. These findings agree with those of Igwe *et al.* (2016) and Coda *et al.* (2012), who reported increased flavonoid and phenolic contents in kunu and other fermented beverages, respectively.

CONCLUSION

Kunu offers significant nutritional benefits that can be further enhanced by mono- and co-cultural fermentation, particularly when using *S. cerevisiae* and *L. plantarum*, rather than relying on natural fermentation alone. Mono- and co-cultural fermentation increase the protein, ash, flavonoid and phenolic contents of kunu while reducing the fat content, which is beneficial for human health. Hence, it can be concluded that mono- and co-culturally fermented kunu could serve as a good source of nutrition, as starter cultures are easier to use and offer excellent possibilities for greater control over the natural fermentation process.

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CONFLICT OF INTEREST

There is no conflict of interest.

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