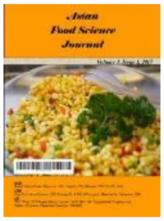

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Effect of *Lactobacillus* Species and *Saccharomyces cerevisiae* on the Mineral and Anti-nutrient Composition of *Kunu* – A Fermented Millet Based Food

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Authors' contributions

This work was carried out in collaboration between authors SAA and OCO. Author SAA designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript .Author OCO managed the analyses of the study, managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The effect of *Lactobacillus* species and *Saccharomyces cerevisiae* on the mineral and anti-nutrient composition of *kunu* samples was investigated. Two species of *Lactobacillus* and yeast were used as starter cultures for the fermentation of *kunu* which was allowed to ferment for 48 hours at 35±2°C. The mineral and anti-nutrient content were analysed using standard procedure. Results obtained showed that nitrogen, calcium, potassium and magnesium content of *kunu* fermented using starter culture were higher than that of naturally fermented *kunu*. The highest mineral content in terms of nitrogen (0.97 mg/100 ml), potassium (67.57 mg/100 ml), magnesium (51.33 mg/100 ml) and calcium (11.02 mg/100 ml) were detected in *kunu* fermented using *S. cerevisiae* and *L. plantarum*. However, the amount of phytate, oxalate and tannin contents were lower in *kunu* fermented using starter culture compared with naturally fermented *kunu*. The least phytate (40.00 mg/kg), tannin (24.03 mg/kg) and oxalate content (15.00 mg/kg) were reported in samples with *S.*

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cerevisiae and *L. plantarum*. In conclusion, the co-culturally fermented *kunu* of *S. cerevisiae* and *L. plantarum* could play an important role reducing the level of anti-nutrient and also increase the mineral composition of *kunu* compared to natural or spontaneous fermentation.

Keywords: Kunu; beverage; millet; anti-nutrient; mineral.

1. INTRODUCTION

Kunu is a non-alcoholic beverage commonly consumed among less affluent people in Nigeria. It is more nutritious than the carbonated beverages, thus enhance daily nutrient intake of consumers. *Kunu* production involves sorting, cleaning, steeping, wet milling, wet sieving, settling and decantation [1] which is usually prepared from cereal grains like millet, sorghum and maize locally by women at house-hold level. It can be flavoured with spices like ginger, black pepper, and cloves to improve its organoleptic properties [2].

Fermentation process contributes immensely to the improvement in shelf-life and nutritional value of fermented foods [3]. It inhibits the growth and survival of pathogenic microorganisms, also the toxin produced from the microorganisms [4]. The major inhibitory effect of fermentation is the production of organic acids, mainly lactic acid by the fermenting microorganisms and this depends on the types of microorganism involved, the temperature, the amount of un-dissociated acid and the buffering capacities of the food [5].

Anti-nutrients such as phytate, oxalate and tannin are produced in *kunu* can decrease the absorption of minerals such as zinc, calcium and potassium which may lead to mineral deficiency. These deficiencies pose a major concern on the health condition of an individual especially in the developing countries where *kunu* serves as part of the major diet.

However, certain traditional food processing techniques such as fermentation has been reported to cause improvement in the contents of certain essential amino acids, vitamins and reduction in anti-nutrients in some fermented foods [6].

Therefore, this study is designed to evaluate the effect of *Lactobacillus species* and *Saccharomyces cerevisiae* on the mineral and anti-nutrient composition of *kunu*- a fermented millet based food.

2. MATERIALS AND METHODS

2.1 Collection of Sample

Raw millet grains (*Pennisetum glaucum*) was purchased at Oja-oba market, Akure using well labelled sterile transparent polyethylene bags which was immediately transported to the Microbiology laboratory, Federal University of Technology, Akure for processing into *kunu*.

2.2 Source of Starter

Pure cultures of identified and characterised *Lactobacillus plantarum, Lactobacillus fermentum* and *S. cerevisiae* isolated from *kunu* were used as starter cultures for the fermentation process. Ten millilitres (10 ml) each of the sample were homogenized in 90 ml sterile peptone water solution to form the stock cultures. One millilitre (1.0 ml) from the stock culture was pipette and serially diluted to appropriate dilutions of 10^{-5} and 10^{-3} for bacteria and yeast respectively. de Man's Rogosa Sharpe agar (MRSA) and yeast extract agar were prepared according to manufacturers' guidelines and used for culturing the lactic acid bacteria and yeast isolates respectively. Yeast plates were incubated at 37°C for 24 hours and MRS agar plates were incubated anaerobically at 28°C for 24-48 hours. The pure isolates were obtained by repeated streaking on freshly prepared microbiological media. Characterization and identification of the isolated microorganisms were based on their cultural, microscopy and biochemical tests [7].

2.3 Inoculum Preparation

Three hundred millilitres (300 ml) of pasteurized *kunu* was prepared into different fermenting vessels. Bacterial suspension of $2.0x10^2$ cfu/ml and $2.0x10^2$ sfu/ml of yeast suspension each were used as starter cultures for the fermentation process at ambient temperature for 48 hours [8].

2.4 Preparation of *Kunu*

The method of Ayo et al. [9] was used in the production of *kunu* (Fig. 1). Two kilogram (2 kg) of cleaned millet grains was washed and steeped in sterile water for 72 hours to soften the seed. The grains were washed, wet milled along with added spices and then sieved in which the supernatant was decanted to obtain clear slurry. The slurry was divided into two unequal parts; two-third (75%) was added to boiling water, stirred and cooled to a temperature of 35±2°C subsequently, one-quarter (25%) was added. The mixture were thoroughly stirred and sweetened with 10% granulated sugar. The product obtained (*kunu*) was divided into two portions; portion one was packed into plastic bottles and pasteurized at 75ºC for 30 minutes and then allowed to cool, while the other portion (unpasteurized) was also packed into a plastic bottle for natural fermentation for 48 hours.

2.5 Mineral Composition

The mineral composition of *kunu* starter culture and naturally fermented *kunu* were determined described by Barnett and Hiter, [10] and the one modified by Asuquo et al. [11]. The parameters monitored include nitrogen, calcium, potassium, magnesium and sodium contents.

2.6 Antinutrient Composition

The antinutrient composition of *kunu* from starter culture and naturally fermented *kunu* were monitored according to AOAC [12]. The parameters monitored include phytate, oxalate and tannin content.

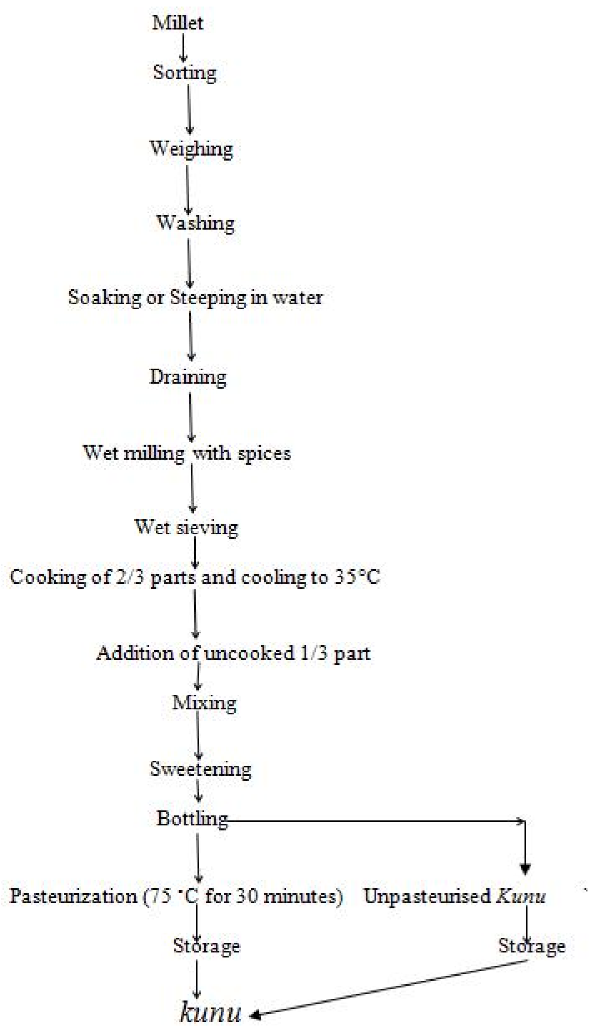

Fig. 1. Processes involved in *kunu* production
Source: [9]

2.7 Statistical Analysis

The data obtained were analyzed. Data are presented as mean ± standard error. Significance of difference between different groups was tested using one-way analysis of variance (ANOVA) using SPSS Window 8 Version 23 software.

3. RESULTS

3.1 Mineral Composition of *Kunu* Starter Cultures

Table 1 shows the mineral composition of *kunu* fermented using starter cultures. Nitrogen (0.97.00 mg/100 ml), calcium (11.02 mg/100 ml), magnessium (51.33 mg/100 ml) and potassium (67.57 mg/100 ml) were highest in sample fermented using *S. cerevisiae* and *L. plantarum* compared with naturally fermented *kunu.*

3.2 Tannin Composition of *Kunu* Fermented Using Starter Cultures

Fig. 2 shows the tannin composition of *kunu* fermented using starter cultures. The result showed considerable reduction in tannin content of *kunu* fermented using starter culture compared with naturally fermented *kunu*.

The least tannin content (24.03 mg/kg) was obtained in the sample inoculated with *Lb. plantarum* and *S. cerevisiae* compared with naturally fermented *kunu* 42.00 mg/kg.

Table 1. Mineral composition of *kunu* fermented using starter cultures

Isolates	Nitrogen (mg/100 ml)	Calcium (mg/100 ml)	Magnesium (mg/100 ml)	Sodium (mg/100 ml)	Potassium (mg/100 ml)
SAC+LFT	0.80±0.00^{c}	10.89±0.11^{e}	49.96±0.04^{d}	18.00±0.00^{b}	67.00±0.00^{e}
SAC+LPT	0.97±0.01^{d}	11.02±0.01^{f}	51.33±0.33^{e}	16.80±0.01^{a}	67.57±0.01^{e}
LPT+LFT	0.94±0.00^{d}	10.12±0.01^{d}	46.00±0.00^{c}	23.00±0.00^{e}	65.07±0.33^{d}
SAC	0.62±0.00^{b}	7.35±0.01^{c}	43.00±0.00^{b}	21.00±0.00^{d}	60.01±0.01^{b}
LPT	0.60±0.00^{b}	7.30±0.00^{b}	41.01±0.01^{b}	19.01±0.01^{c}	62.00±0.01^{c}
LFT	0.61±0.00^{b}	7.30±0.01^{b}	40.00±0.00^{b}	18.06±0.03^{b}	60.67±0.67^{b}
NFM	0.50±0.00^{a}	1.13±0.00^{a}	22.99±0.00^{a}	30.01±0.00^{f}	50.46±0.00^{a}

Values are means of triplicate determinations ± Standard error. Means in the same column with different superscripts are significantly different (P≤0.05).

Key: SAC = S. cerevisiae; NFM= naturally fermented kunu; LPT= Lb. plantarum; LFT= Lb. fermentum; SAC+ LFT= S. cerevisiae, Lb. fermentum; SAC+LPT= S. cerevisiae, Lb. plantarum; LPT+LFT= Lb. fermentum, Lb. plantarum.

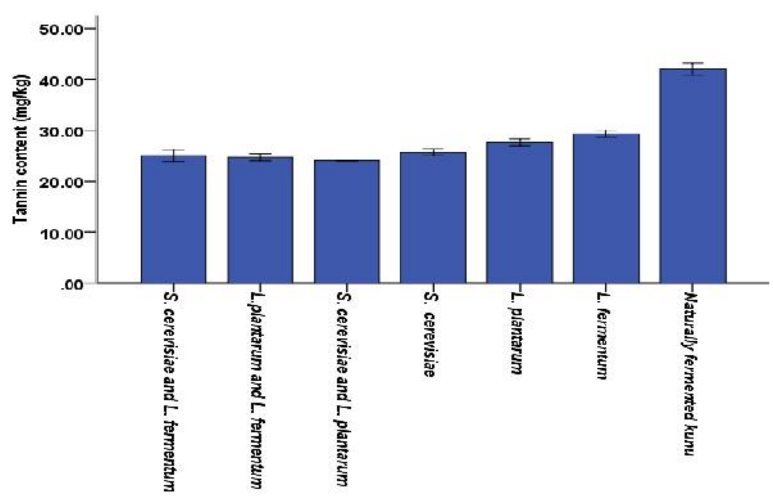

Fig. 2. Tannin composition of *kunu* fermented using starter cultures

3.3 Oxalate Composition of *Kunu* Fermented Using Starter Cultures

Fig. 3 shows the oxalate composition of *kunu* fermented using starter cultures. The oxalate content of *kunu* fermented using starter cultures decreased compared with naturally fermented *kunu.* The lowest oxalate content value (15.00 mg/kg) was obtained in the sample inoculated with *Lb. plantarum* and *S. cerevisiae*) compared with naturally fermented *kunu* (67.00 mg/kg).

3.4 Phytate Content of *Kunu* Fermented Using Starter Cultures

Fig. 4 shows the phytate content of *kunu* fermented using starter cultures. The result showed decreased in phytate content of *kunu* fermented using starter cultures compared with naturally fermented *kunu.* The least phytate content (40.00 mg/kg) was obtained in the sample inoculated with *Lb. fermented* and *S. cerevisiae* compared with naturally fermented *kunu* (97.67 mg/kg).

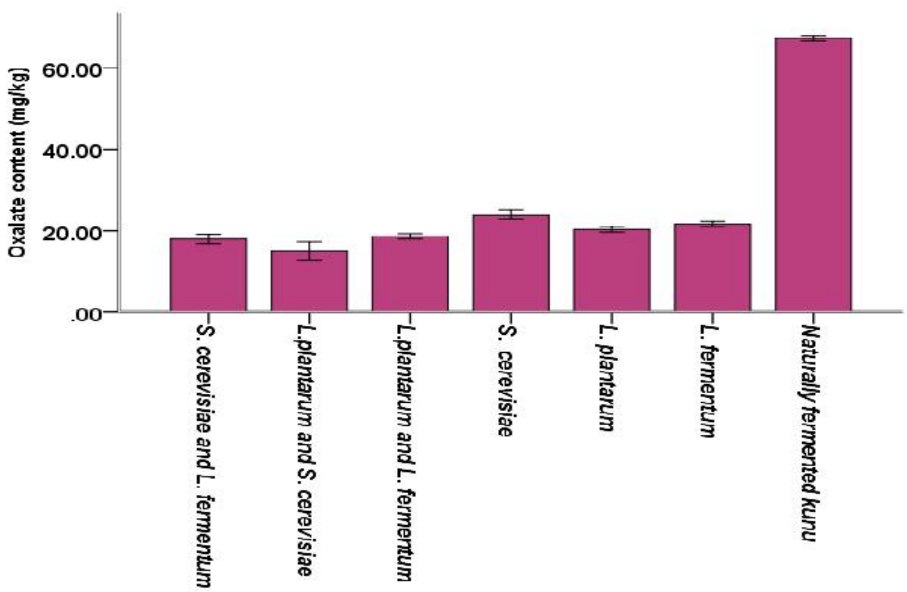

Fig. 3. Oxalate content of *kunu* fermented using starter cultures

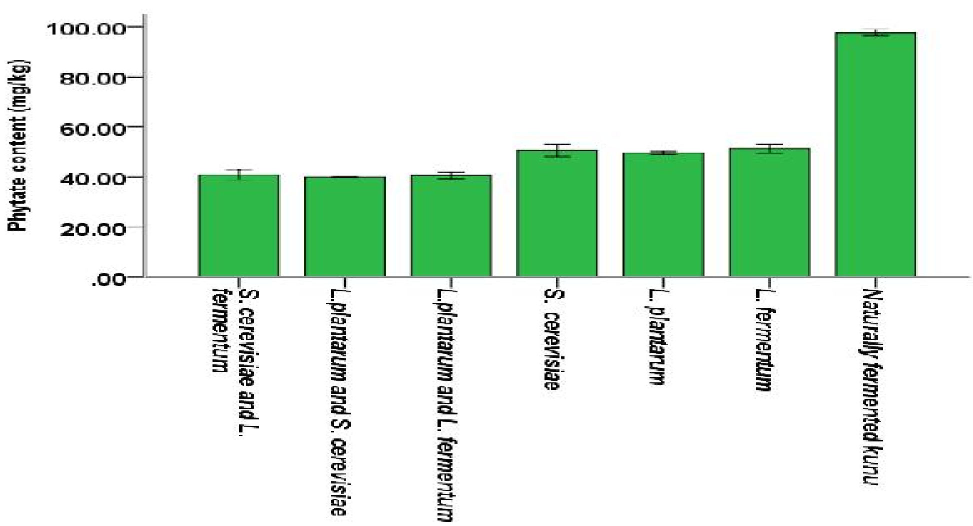

Fig. 4. Phytate content of *kunu* fermented using starter cultures

4. DISCUSSION

The effect of mineral elements and anti-nutrient in food cannot be overemphasized. Minerals are essential for health and involved in cellular function of human body. They can also form an integral part of enzyme or protein structure. They are vital for normal growth, maintenance, effective immune system and prevention of cell damage [13]. The result from the study showed an increase in the mineral content such as nitrogen, calcium, potassium, and magnesium of *kunu* fermented using starter cultures compared with naturally fermented samples. The significant increase in the nitrogen, potassium, calcium, and magnesium content in some of these fermented samples may be adduced to the degradation of anti-nutritional factors like phytate, oxalate and tannin by the fermenting microorganisms thereby releasing these minerals during fermentation [14]. This result obtained is similar to the report of Adegbehingbe, [15] who observed an increase in mineral of ogwo, fermented sorghum–Irish potato gruel using starter cultures. This is in agreement with the work of Olaoye et al. [16] who observed an increase in mineral content of magnesium, calcium and potassium during fermentation of fortified *kunu*.

Anti-nutrients like phytate, tannin and oxalate present in *kunu* are natural or synthetic compounds that interfere with the absorption of nutrients. The report from this finding indicates decrease in tannin, oxalate and phytate content of *kunu* fermented using starter cultures compared with naturally fermented *kunu*. The decrease in tannin content of *kunu* fermented using starter cultures compared with naturally fermented *kunu* may be as a result of the processing that the sample was subjected to couple with the activities of microbial enzyme involved in the fermentation [17]. This is in agreement with Igwe et al. [18] that reported decrease in tannin content of *kunu*. Obizoba and Atii [19] reported a decrease in tannin content of pearl millet during fermentation. Usha and Chandra [20] also reported a reduction in tannin content of finger millet fermented using starter culture.

Moreso, the report from this result showed a decrease in the phytate content of *kunu* fermented using starter cultures compared with naturally fermented *kunu*. The decrease in the phytate content of *kunu* fermented using starter culture could be as a result of possible secretion of the enzyme phytase by the microorganisms into the fermenting sample. This enzyme is capable of hydrolyzing phytate thereby decreasing the phytate content of the fermenting sample [21]. It had earlier been reported that lactic acid bacteria could hydrolyse phytate thereby releasing minerals which are bound to it [22,15]. *S. cerevisiae* has been reported to reduce phytic acid in water melon rinds [23]. This result obtained is similar to the report of Adegbehingbe [15] who reported a decrease in phytate contents of ogwo, fermented sorghum–Irish potato gruel using starter cultures. Mulimani et al. [24] also reported a 1/3 reduction in the phytic acid content of soybean due to fermentation. Besides, combination of *Lb. plantarum* and *Leuconostoc* strains with the *S. cerevisiae* strains had been reported to enhance phytate reduction in fermented Sough dough bread [25].

The result obtained also showed a decrease in the oxalate content of *kunu* fermented using starter cultures compared with naturally fermented *kunu*. The decrease in oxalate content of *kunu* fermented using starter cultures compared with naturally fermented *kunu* could be due to the activities of some microorganisms involved during the fermentation process. The result obtained is in agreement with the Adegbehingbe [15] who reported a decrease in oxalate using starter culture on ogwo sorghum based food. The reduction in oxalate levels in the starter fermented samples alleviates the fear of renal calcium absorption which results into kidney stones which is characteristic of food with high oxalate contents [26].

5. CONCLUSION

Microbial fermentation demonstrated an efficient method for reducing tannins, phytates and oxalate contents, and also the increase in mineral composition of fermented foods.

The present research work has shown that co-cultural fermentation of *S. cerevisiae* and *L. plantarum* plays an important role in detoxifying the above mentioned anti-nutrients and causing an increase in the mineral composition of *kunu* compared to natural or spontaneous fermentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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