Microbial Dynamics and Preservation of Kunu Drink

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Kunu is a fermented non-alcoholic beverage consumed all over Nigeria. The drink is served as an alternative to alcohol due to its perceived extreme nourishing and therapeutic properties. Varieties of this beverage are determined mostly by the type of grain, the supplements, sensory additives used, and the process employed during its production. Dietary quality is paramount in nutritional well-being and a key factor in human overall health development. The nutritional quality of grains utilised for *Kunu* production makes the drink more appealing to a large growing population when compared to some other drinks. Some use *Kunu* drink as an infant weaning drink, thus serving as a priming beverage for infants due to its rich probiotic and nutritional properties.

Kunu beverage fermentation diet priming

1. Introduction

Kunu, a fermented non-alcoholic beverage, is popular in the northern region of Nigeria. Furthermore, its refreshing nature increased the popularity of the beverage to non-alcoholic drink lovers [1] in many other parts of Nigeria. Varieties of this non-alcoholic beverage are determined mostly by the substrates (major grain used), supplements or sensory attributes employed during its production and primarily prepared to suit each individual's desire as porridge or free-flowing gruel [2][3]. Foods produced from single or mixed cereals can be taken by people of all ages and social classes, serving as breakfast for some adults, weaning foods for babies and beverages for people living in different local African communities [4][5]. These beverages have been produced from different grains such as *acha* (*Digitalis exilis*), sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), maize (*Zea mays*), millet (*Penisetum typhoides*) and wheat (*Triticum aestivum*). Beverages of African origin are produced from different raw materials including cereal grains, flowers, milk, plant juices, legumes and fruits [6]. They vary due to their origin, compositions and processing techniques particular to the cultural or ethnic groups that produced them [7][8]. Supplements used in producing these beverages improve their protein and amino acids content and their antioxidant properties [2][9].

2. Pre-Fermentation in Kunu Production

Wide varieties exist in the protocols of preparation, which impact the taste, cultural norms and habits around *Kunu* [10][11]. The pre-fermentation in *Kunu* production begins with cereal sorting, washing and steeping alone or in combination with additives and spices. The Steeping process can either be via the replacement of water at different time intervals or without water replacement [12][13][14]. Steeping is primarily employed to soften and increase grains' moisture content thereby easing milling. Certain physicochemical and biological changes (such as swelling of the grain, degradation of soluble carbohydrates and removal of some pigments, microorganisms and bitter substances) occur during steeping [15][16]. The product's characteristic organoleptic properties and nutrition components are due to the spontaneous fermentation mediated primarily by bacterial species during the steeping stage [17][18][19]. Furthermore, the highly fermentable solutes in the grains are transferred into the steeping water, while enhancing the release of sulphites, free reducing sugars and temperature level, which aids the rapid development of bacteria [16] needed during fermentation. Although the microbial community dynamics during the steeping and pre-fermentation stage and process are dependent on the type and variety of grains used [2][3], firmicutes dominate most of the steeping and souring stages during the processing of all millet varieties [20]. In a throughput metagenomic analysis of microbial dynamics during the steeping stage in *Ogi* production [20] an increase in the R and diversity (Shannon–Wiener index, H′) of ASVs at 48 h was observed when compared with 12 h of steeping; and that throughout the steeping stage, R and diversity (Shannon–Wiener index, H′) of ASVs were fluctuating among millet varieties [20]. They also reported the dominance of firmicutes during the steeping and souring stages of all three millet varieties. Additionally, another report [3] confirmed the prevalence of firmicutes in most of the processing stages, as proteobacteria dominated the rest of the stages. Furthermore, they observed the dominance of *Lactobacillus* at the genus level during most stages of processing and in the final product of the two *Kunu* formulations [3]. A similar observation of *Lactobacillus* spp. dominance in *Kunu-zaki* has been reported by others [21][22]. The genera *Lactobacillus*, *Lactococcus*, *Pediococcus and Burkholderia-Caballeronia-Paraburkholderia* were reported by Chibuzor-Onyema [20] to be present at most steeping and souring stages of all three millet varieties used. *Lactobacillus, Lactococcus* and *Saccharomyces* spp. have been implicated during the steeping stage [3]. However, Oyeyiola [23] reported the presence of bacteria (*Pediococcus pentosaceus* and *Lactobaclllus plantarum*), moulds (*Aspergillus versicolor*, *Penicillim nigricans* and *Rhizopus stolonifer*) and yeast (Saccharomyces cerevisiae) with the bacteria and yeast persisting to the end of the steeping period. Similar findings have shown that *Lactobacillus fermenturn*, L. *leichmannii*, *Bacillus subtilis*, *Enterobacter aerogenes* and *E. cloacae* dominated throughout the production process, with the latter disappearing during the post-fermentation stage [24]. Growth of *Saccharomyces cerevisiae* became apparent with increased acidity during the post-fermentation stage.

3. Post-Fermentation in Kunu Production

The dynamics of microbes during the post-fermentation stage of *Kunu* production are quite interesting as certain microbes observed in the pre-fermentation are eliminated during this stage. Moulds are eliminated prior to post fermentation and after steeping there was a unilateral in the ratio of Gram positive to Gram negative after the steeping stage [24]. Other reports [3] revealed that different bacterial succession patterns at different stages of fermentation of three different *Kunu* formulations. Some investigators [25] implicated *S. rouxii* and *S. cerevisiae* as being partly responsible for fermented maize organoleptic properties, [26] and highlighted *Penicillium* as the major organisms responsible for the fermentation and nutritional improvement of cereal-based fermented foods (*Ogi* and *Kunu-zaki*). However, overall the fermentation stages of *Kunu* production are driven by a cocktail of notable yeasts such as *Saccharomyces cerevisiae* [27] and predominantly lactic acid bacteria [28][29][30]. There is a relatively low

OTU (operational taxonomic unit) diversity at the start of the post-fermentation in all the *Kunu* formulations due to hot water (100 °C) treatment prior to gelatinisation of the milled grains [3]. The bacteriostatic and/or bactericidal effect of such heat treatment on the indigenous microbiota might have contributed to the reduction in species diversity [31]. The total viable count of microbiota reduced sharply following heat treatment of fermented maize (*Ogi*) at 100 °C [32]. Generally, *Kunu* fermentation is seen as a lactic acid fermentation type, with slightly acidic pH of about 6.0 ab initio which reduces to an acidic pH of about 4.76 at the end of 12 h, and then decreases further to 3.0 in the final *Kunu* product [9][27]. Reduction in the pH gives a competitive advantage to certain microbial communities [33][34]. An increase in OTUs between the onset and first 6 h of fermentation might be due to the moderate pH and other favourable conditions (e.g., temperature, oxygen availability, absence of growth inhibition metabolites) during this period, which caused the recovery of microorganisms suppressed by the heat treatment in the gelatinisation stage [35]. However, as the selection pressure increases with further pH reduction toward the end of fermentation (at 12 h), only adapted species (mostly lactic acid bacteria) proliferate [27]. It is important to note again that different substrate types play critical roles not only in the balance of biochemical reactions and conditions (such as pH, oxygen availability, redox reactions, enzymatic activities and temperature), but also the genetic and functional diversity and dynamics of microbiota during fermentation [36][37][38]. From different research findings, Lactobacilli appears to be dominant during post-fermentation and it is responsible for the acidity in cereal fermentation [3][13][22][25][39][40][41]. *Lactobacillus* spp. includes several species with probiotic [42] and physiological capabilities for the digestion of complex polysaccharides in human and animal diets [43]. This may be the reason for some of the nutritional and health benefits generally associated with *Kunu* consumption [5]

Furthermore, the essential metabolites (such as pyruvate, amino acids and vitamins) provided by *S. cerevisiae* during post-fermentation work to excite the growth of *Lactobacilli*, as the yeast also utilises certain metabolites produced by *Lactobacilli* as their carbon sources [44]. Different yeast species have been reported to have pectinase activity which could engender the growth of other microorganisms during fermentation [3]. The antioxidant potential of fermented foods through an increase in the phenolic contents has been linked with the presence of yeasts [45][46]. More so, different studies have revealed cereal fermentation leads to phytase-dependent dephosphorylation of phytic acid. The phytic acid chelates many important nutritional minerals in food, hence its digestion results in essential minerals (such as iron, zinc, calcium and magnesium) accessibility [5]. The phytase activity within the cereal is induced during *Lactobacilli* acidification, thus engendering desirable biochemical changes such as aromatic compounds production, proteolytic and lipolytic activities [47][48][49].

4. Effects of Fermentation on Kunu Production

Fermentation is a procedure which aids the breakdown of complex organic molecules (carbohydrates, proteins, lipids) into simpler molecules via the activities of microorganisms [50]. Application of fermentation in production processes increases the security of food chain supply, hence, denotes a fundamental part of food culture development universally [51]. Furthermore, fermentation is beneficial in preservation of various food products, supplies diverse flavours, enhances taste, acidity, digestibility, texture and notably increases the nutritional potentials of the raw food [52]. Owing to the aforementioned vital characteristics associated with several fermented foods, fermented foods are produced and consumed worldwide [53][54]. Food preservation is of utmost importance, hence, fermentation is seemingly perceived as a tool for achieving the preservation of food throughout Africa and beyond, where current food preservation methods are still not yet fully established [52]. Food fermentation has been found to surge the macro-mineral and micro-mineral bioavailability by decreasing non-digestible material present in plants, including cellulose, hemicelluloses, glucuronic acid and polygalacturonic acids [55]. Fermented foods can be beneficial to consumers. The fermentation of much cereal-related food has overtime shown to have a direct therapeutic influence on the consumers [56], consequently, fermented food promotes consumers' wellbeing via boosting the number of accessible vitamins, including thiamine, folic acid, niacin and riboflavin [57]. Fermented foods, compared with simple foods regarding peptide production, antioxidants, organoleptic and probiotic properties as well as antimicrobial activity, have shown to be more acceptable, as they aid in the reduction of anti-nutrients and toxins [50]. Digestibility is an essential factor in food nutritional value, but other vital nutrients present could, however, be enriched through the fermentation process. This is obvious in regard to the variations in nutrient composition and their biochemical qualities relative to the initial constituents [50]. Fermentation has been proven to reduce the required energy for cooking and produce harmless products [58][59]. In addition, fermentation has been documented to enhance the iron utilisation by the breaking down of complex nutrients into inorganic iron and vitamin C [59]. According to Erkmen et al. [60], carbohydrates with non-digestible oligo- and polysaccharides' levels are largely reduced by natural fermentation and thereby, this boosts vitamin B group accessibility, improves amino acid synthesis and increases enzymatic degradation of phytates through providing ideal pH conditions existing in a composite form with polyvalent cations including iron, zinc, magnesium and calcium [59]. According to Singh et al. [61], cereals crops (namely: maize, millet, sorghum and other grains) promote the amount of protein as well as the levels of lysine when they undergo the fermentation process [62]. Hydrolytic enzymes have been reported to play a vital part in pre-fermentation [63]. Hydrolytic enzymes increase the nutritional and sensory quality of the *Kunu* product. Several researchers have used hydrolytic enzymes to produce *Kunu-zaki* which increased the nutritional and sensory quality of the product [64][65][66].

5. Malting in Kunu Production

Malting has been reported to primarily promote the synthesis of hydrolytic enzymes capable of solubilising large macro molecules into low molecular weight compounds [67][68]. Whereas fermentation favours the growth of microorganism of importance and prevents the growth of harmful microorganisms by increasing the acidity, thus preserving the food [69][70][71], malting enhances the bio-accessibility of minerals due to an increase in phytase enzyme activity which leads to phytate content reduction during grain sprouting [71][72]. During malting and fermentation, the microbial community implicated are yeasts and LAB, which raise the titratable acidity, reduce the pH and clog the development and proliferation of microbial contaminants such as coliforms and enterobacteriaceae, hence, extending the shelf-life of the cereal product [73]. During cereal drying and malting, reports [74] show that there is a reduction in the total microbial count due to the dried state of the cereal. However, it has been observed [72] that although the total plate count (TPC) decreased from 5.69 log Cfu/g in non-malted finger millet to 5 log Cfu/g in malted finger millet flour, LAB increased from 4.66 log Cfu/g to 6 log Cfu/g [74]. The reason for this increase might be due to the increase in the amount and availability of soluble sugars during malting as a result of extensive starch degradation [75]. The increase in the digestible and fermentable sugars after malting in *Kunu* production can impact the quality and amount of fermentative microbes necessary for the improved probiotic characteristics associated with the *Kunu* product. Probiotic bacteria alleviate digestive distress, reduce cholesterol levels, stall the ageing process and enhance energy and improve immunity [41][76]. Other authors [77] reported that LAB (*Lactobacillus fermentum*, *Lactobacillus salivarius*, *Pediococcus* and some species of *Leuconostoc*) are the primary microorganisms usually found in malted and fermented finger millet flour. LAB has been implicated in the induction of vitamin C and minerals synthesis and assimilation in the body [78]. Additionally, malting has been reported as a useful processing treatment which enhances the nutritional quality of the cereal product [6][79]. Grains within a range of time increase hydrolytic enzyme activities, thus improving the contents of certain essential amino acids, total sugars, and B-group vitamins, and reducing its dry matter, starch and anti-nutrients. Other investigators [79][80] opined that the partial hydrolysis during malting enhances the digestibility of storage proteins and starch. Furthermore, an improved taste of *Kunu-zaki* formulations due to malting of the pearl millet used has been reported [78]. Additionally, Akoma et al. [62] highlighted the influence of malting on the nutritional characteristics of *Kunu-zaki.* They observed that malted cereals improved the nutritional quality of the *Kunu-zaki* and elevated lymphocyte counts in the blood samples of animals fed with *Kunu-zaki*.

6. Effects of Malting on Kunu Production

Malting is a procedure usually employed mostly in West Africa towards formulating conventionally non-alcoholic beverages, beers and other varieties of food stuffs [81]. Malting as a process has been shown to increase the diastatic activities and protein content of samples [82]. Furthermore, malted cereal grains could bring about biochemical changes and yield malt alongside enhanced dietary value that could be utilised in diverse traditional recipes [83]. In line with the aforementioned report, [84] reported it has been demonstrated that malting significantly enhanced the in vitro starch (86% to 112%),protein (14% to 26%) and digestibility in pearl millet, and the enhancement by germination was expressively greater when compared with blanching [84]. An upsurge in tryptophan, lysine and non-protein nitrogen has also been detected [83]. Malting also had a significant effect with respect to the carbohydrate and fat contents of samples compared with non-malted samples [14].

It has been shown [85] that after the period of germination, steeping, debranning, and dry heating, protein digestibility is usually improved. This can, however, be ascribed to the decrease of anti-nutritional factors including tannins, polyphenols and phytic acid, which form complexes when interacting with protein. Malting, however, involves stages such as soaking, sprouting and drying, tailored to changing cereal grains into malt with significant enzymes and nutrient compositions [86]. Steeping involves soaking the grains in water or a different liquid to soften, cleanse, and extract some vital constituents in the grains [87]. The seed absorbs water during the steeping period, which results in swelling up of the seeds and thus brings about sprouting. However, this leads to a significant increase in vitamins content and a decrease in tannins and total phenols content. A study by [81] revealed a substantial difference in anti-nutrients, proximate, functional and mineral properties when steeped in lime and plantain peel ash solution before 24 h of sprouting of pearl millet at different steeping times. It has been found that the incorporation of ground-malted rice into millet in *Kunu-zaki* production was largely desired in both aroma and taste and was found to be remarkably discrete from the other products. Additionally, malted cereals enrich the nutritive value of the *Kunu-zaki*, and this has been reported to elevate the lymphocyte counts found in the blood samples of animals fed with it. This, however, reveals its therapeutic features, a notion generally preferred by several consumers [88]. Furthermore a laboratory trial [88] proved that addition of ground-malted rice and millet during the production of *Kunu-zaki* was more nourishing (crude protein; 0.74%; crude fat: 0.50%; iron: 50 ppm; calcium: 88 ppm) than the other products, and an albino rat used as a model gained more weight when compared with the control. Nonetheless, various minerals could vanish, and this occurrence may apparently be linked to solubilisation and leaching during steeping [81]. This nutritional content decrease could be likened to the loss of molecular weight of nitrogenous compounds during the process of steeping and rinsing of the millet grains and hydrolysis of lipid and oxidation of fatty acids during the time germination [89]. Variations are usually observed in the nutritional values of grains after germination, this could be ascribed to the usage by growing sprouts. Furthermore, dietary compositional variations resulting from sprouting are frequently concomitant with health benefits [90][91]. Based on laboratory trials, it has been revealed that the extraction and bioaccessibility of minerals including zinc, iron and calcium were enhanced in finger millet and pearl millet by germination; however, the anti-nutritional constituents such as phytic acid were deceased [92][93][94]

7. Preservation and Shelf-Life Extension

The shelf instability of *Kunu* is one of the bottlenecks to its large-scale production [95]. This lack of shelf stability could be also due to technology of production [96], such as poor hygienic environments and conditions; poor raw materials and ingredients handling [97]. Microbial invasion of *Kunu* during the processing activities (such as water, material handling and preservation additive) has also been highlighted by [95]. Currently, efforts are increasing in the modification of *Kunu* beverage processing with a view to enhancing its nutritive value, shelf-life and other possible therapeutic qualities. Most of the pathogens associated with food materials cause diarrhoea [98]. One of the fundamental reasons leading to the proliferation of these microbes in *Kunu* is the lack of public health regulatory agency to monitor the production processes of *Kunu* drinks despite the food-related poisoning associated with it [98]. A report [99] suggests that local drinks such as *Kunu* are possible mediators for zoonotic and food-borne infections such as *Staphylococcosis*, *Salmonellosis, Brucellosis*, *Tuberculosis*, *Shigellosis*, *Listeriosis,* etc. [99]. Additionally, microbes such as *Salmonella*, *Clostridium*, *Bacillus* and *Staphylococcus aureus*, *Pseudomona aeruginosa*, *Vibrio cholera* and *Escherichia coli* might contaminate food and cause physical and nutritional quality problems [100].

However, shelf-life improvement and food-desirable properties enhancement (increased nutritional value, palatability) could be possible due to fermentation involved in the production of *Kunu* [101]. Muyanja and Namugumya [73] postulated that the microbial community implicated (yeasts and LAB) during malting and fermentation raises titratable acidity, thus, reducing the pH in *Kunu* beverage. This aids in the inhibition of the proliferation of microbial contaminants such as coliforms and enterobacteriaceae, and in extending the shelf-life of the cereal product. Lactic acid fermentation helps improve the shelf-life of fermented products via the production of different antimicrobial metabolites during fermentation [102]. LAB produce technologically important metabolites, such as ethanol, hydrogen peroxide, diacetyl, bacteriocins, aromatic compounds and exopolysaccharides. These in combination with weak acids can reduce the pH of the environment [103][104][105][106][107][108] making it unfavourable for the growth of undesirable and pathogenic microorganisms [101][109]. Other workers suggested that adding refrigeration and preservatives can improve the shelf stability of the *Kunu-zaki* product, which should be refrigerated rather than stored at ambient temperature to increase the shelf-life. Alternatively, Fapohunda et al. [110] postulated that the sensory and keeping quality of *Kunu-zaki* might be enhanced by the addition of ginger solution concentrated in a certain proportion aiding improvement in the shelf-life and storage optimisation. In another report, [13], it was noted that ginger extract concentrate (GEC) added into processed *Kunu-zaki* at different concentrations enhanced the shelf-life of the *Kunu* samples for 48–72 h.

The method of drying the beverage may also have an impact. Different researchers [111][112] have reported freeze-drying and tray-drying methods to enhance *kunu-zaki* storage without affecting its nutritional, sensory and physical properties throughout the period of storage. Although Chaven et al. [113] highlighted that drum-drying destroyed heat-sensitive nutrients (such as lysine) in *Kunu*, Cook [114] reported that dehydration of Kunu by drum- or tray-drying helped to prolong its shelf-life.

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