Growth and Survival of *Escherichia coli* in Kunun Zaki During Storage

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**Abstract:** Growth and survival of *Escherichia coli* counts were determined in Kunun-zaki along with physio-chemical analysis parameters. The Kunun-zaki samples were stored at -4°C, 5±1°C and at room temperature (28±2°C) for 5 days. There was a decline in total bacterial count from 2.52x10⁶ cfu/ml to 8.50x10⁵ cfu/ml and 3.00 x 10⁵ cfu/ml of Kunun-zaki stored at 28±2°C and -4°C, respectively. pH values decreased from 5.10 to 3.01 and 4.29 with corresponding increase in titratable acidity values from 2.50 ml to 8.40ml (0.1N NaOH) for the Kunun-zaki stored at 28±2°C and -4°C, respectively. In the *E. coli* inoculated Kunun-zaki, *E. coli* survived up to 1.21 x 10⁶ cfu/ml on day 5 in the -4°C storage temperature. At this storage temperature, pH decreased gradually from 5.98 to 4.66 with a corresponding gradual increase in titratable acidity from 1.00ml to 4.10ml (0.1N NaOH). The study showed that storage temperature is critical to the growth and survival of *E.coli* in the fermented Kunun-zaki.

**Key words:** Kunun-zaki  •  *Escherichia coli*  •  Survival and pH

**INTRODUCTION**

Kunun-zaki is a traditional fermented non-alcoholic beverage widely consumed in Northern Nigeria. It can be produced from millet, sorghum or maize. It has an immense social, economic, nutritional and medicinal benefits to the numerous consumers [1-3]. Kunun zaki, like other locally made drinks are widely consumed in Nigeria. In most Nigerian cities, the sales and consumption of this locally made beverage is high due to the highest cost of other non-alcoholic drinks. The non-alcoholic nature of this drink made it to be readily consumed by both Muslims and Christians alike as substitute for alcoholic ones [4]. The drinks is usually hawked in the motor parks, school premises and market places and even served during social gatherings [5].

The traditional production of kunun-zaki is still at village technology level. The process of production involves wet milling of grains, wet sieving, partial gelatinization of the slurry, sugar addition and bottling [6]. The fermentation process may last for 12-72 hours [2], after which it is kept for acidification to develop. Under the processing conditions kunun-zaki can be contaminated from handlers or equipment used. However acidic foods such as kunun-zaki have been considered intrinsically safe due to their low pH and high acidity [1, 7]. Food-borne pathogens such as *Escherichia coli* have been implicated in food poisoning resulting from the consumption or non alcoholic beverages. Osuntogun and Aboaba [4] reported that kunun-zaki is highly prone to microbial deterioration if not adequately stored.

A large number of lactic acid bacteria, moulds and yeasts caused spoilage as they can use the carbohydrate content of foods for fermentation processes producing undesirable changes in the food. The presence of large numbers of the microorganisms could be attributed to the unhygienic conditions of preparation, use of raw materials and utensils contaminated by micro flora. The micro flora of finished product depends on the processing and storage conditions. High temperature and lack of refrigeration facilities in most developing countries have led to the inability to produce and store fresh kunun-zaki.

Faecal contamination of water supplies and contaminated food handlers have most frequently been implicated in outbreaks caused by *Escherichia coli* [8]. This organism has been implicated in the consumption of some acidic foods [7]. Massa et al. [9] reported that *Escherichia coli* may be tolerant to the acidic condition found in yoghurt. Observations have shown that *Escherichia coli* inoculated into acidic foods may remain viable for a few hours at a favourable temperature and up to 1-8 days while refrigerated. The implication is that
kunun-zaki can pose a health risk if the kunun-zaki is poorly handled and recontaminated. The aim of this study is to investigate the growth and survival of *Escherichia coli* in kunun-zaki stored at different storage temperatures.

**MATERIALS AND METHODS**

**Collection of Samples:** The samples of freshly prepared kunun-zaki were collected from Oregbeni market in Benin City, Nigeria. The samples were packaged in 1000ml sterile plastic bottles and immediately transferred to the Microbiology laboratory for the experiment. From the samples 400 ml each was transferred into three sterile plastic container of 500ml capacity which was washed with 70% ethanol and rinsed twice with sterile distilled water. The three sample containers were stored under different conditions namely-4°C, 5±1°C and Room temperature (28±2°C). The storage was carried out immediately to avoid further fermentation processes and contamination outside the area of production.

The samples were monitored for microbial quality, pH changes and titratable acidity at every 24 hours for a period of five days.

Viable counts of bacteria and *E. coli* were determined by pour plating method using Nutrient agar and Eosin methylene blue (EMB) agar, respectively. The samples were serially diluted and 1ml of appropriate dilution was used to inoculate each of the plates in duplicates. The culture plates were then incubated at 37°C for 24-48 hour and colonies counted on a Gallenkemp colony counter.

Growth and survival of *E. coli* was determined in the fermented kunun zaki. The different storage conditions were employed. In each samples, 1.50 x 10³ cfu/ml of 24hours culture of *E. coli* was inoculated.

In all the samples, viable counts for *E. coli* were determined by plating on EMB agar. Changes in pH were measured in these samples using Jenway 332 model pH Meter and Titratable acidity were monitored using titration method. These were determined every day for a period of 5 days.

**RESULTS**

The results on the survival of bacterial loads of the different storage conditions are presented in Fig.1. Bacterial population was decreased considerably from 2.52 x 10⁶cfu/ml to 5.80 x 10⁶cfu/ml (Fig. 1) when the samples were stored at room temperature. The pH values of kunun zaki during the storage period decreased from 5.10 to 4.29 and 3.01 (Fig. 2) and the production of acid increased the titratable acidity from 2.50 ml to 4.90 ml and 8.40 ml (0.1N NaOH) (Fig. 3), respectively.

Survival of *E.coli* in kunun zaki at different temperature are shown in Fig. 4. The *E. coli* grew from 1.50 x10⁷ cfu/ml to 1.21 x 10⁷, 0 and 0 cfu/ml for -4°C, 5±1°C and 28±2°C storage temperature, respectively. During the period, the pH values of kunun zaki inoculated with *E. coli* declined from 5.98 to 5.16, 4.22 and 3.22, while titratable acidity increased to 4.1, 5.9 and 7.4 for-4°C, 5±1°C and 28±2°C, respectively.
DISCUSSION

The growth and survival of *E. coli* was studied in a traditional fermented kunun zaki. The results of microbiological analysis are presented in Fig. 1. The highest bacterial count obtained from kunun zaki (2.52×10^6 cfu/ml at day 0) may be due to the relatively, high moisture content of this fermented beverage typically allows microbial growth [10, 11]. The possible sources of these organisms in the food samples could be from nose, hand, skin and clothing of handlers, coughing, talking and sneezing produced droplets which could settle on the food during transportation, storage and retailing[3, 12].

The survival of the bacteria during storage decreases as time progresses (Fig. 1). Thus, the decline in the viable population of these bacteria is wholly or partially attributed to the detrimental effect of the low pH environment (Fig.2) and high titratable acidity (Fig.3). Most bacteria do not usually survive in an acidic medium. The recorded pH values of the kunun zaki however, found to be lower than those recorded for yoghurt [13].

When *E. coli* was inoculated in the kunun zaki samples, there was a significant reduction during the storage time (Fig.4). These reduced growth rate in the fermented kunun zaki are attributed to acidification of the kunun zaki. Other mechanisms that might also contribute to the decline in the population of *E. coli* are the production of bacteriocin, hydrogen peroxide and ethanol by the fermentative microorganisms [7].

Contamination of kunun zaki can occur during collection of the grains and preparation, during fermentation and after fermentation. The results of Fig.4 showed that the decline in *E. coli* viability during storage of fermented kunun zaki at different temperature was apparently influenced by the storage temperature.

Thus, *E. coli* survived longer at the storage temperature of-4°C. This maybe due to the fact that fermentation rate is faster at the storage temperature of 28±2°C than-4°C. Much acid will be produced and the medium became unfavourable for the *E. coli* to strive, hence, the decline in the viable cells. At the storage temperature of 5±1°C, *E. coli* resisted the acidic medium until day 4 before there was a complete destruction.

The study showed that microorganisms especially *E. coli* can grow and survive during the storage of kunun zaki at different temperature. The surviving rate is higher when stored at-4°C. During the traditional fermentation of kunun zaki, certain safety measure, like the Microbiological safety should be highly practice. However, when the product is stored at any temperature, will help to promote a safer product to the consumers.

The conclusion will be that the storage temperature is a critical control point in the preservation of kunun zaki that is safe from microbiological hazard. This is especially important when the product is stored at -4°C. This temperature prolongs the survival of the pathogens. Thus, the contaminating level at storage and post fermentation contamination are important risk factors regarding the safety of the drink.
REFERENCES