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Accelerated Fermentation Process of *Kunun-Zaki* (A Nigerian Non-Alcoholic Beverage)

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Abstract

Studies on the possibility of speeding up the acidic-fermentation process of *Kunun zaki* by backslopping (adding back) varying concentrations of a 24 hour old *kunun-Zaki* as mother culture inoculum into fresh sorghum starch paste and which were left to ferment at room temperature (28±2°C) and at elevated temperatures (35°C and 45°C respectively) were carried out. The fermentation rates of the fermenting mixtures were monitored by determining the pH, titratable acidity and microbial load with respect to time. Sensory evaluation of the finished *Kunun-zaki* products was carried out to verify the effects of the altered experimental conditions on the acceptability of the various beverage samples. The results showed that while fermentation at room temperature with no backslops (control) lasted for 12 hours, 11 and 9 hours were the respective fermentation times at 35°C and 45°C. The combinations of various backslopping (30% inoculum) and temperature elevation (to 45°C) led to faster fermentation rate by a reduction of fermentation time from 12 to 4 hours. Fermentation at the elevated temperature (45°C) also led to elimination of the possible bacterial pathogenic mesophillic isolates like *Corynebacterium*, *Lactobacillus sp*, *Streptococcuc sp* and *Bacillus subtilis* and also yielded *Kunun-Zaki* with no altered organoleptic quality ($P \leq 0.5$)

Keywords: *Kunun-Zaki*, fermentation, backslopping, iniculum, titrable acidity, lactic acid bacteria.

1.0 Introduction

The use of microorganism or enzymes to cause desirable biochemical changes that could modify sensory quality, enhances nutritive values, lengthen shelf-stability and enhance toxicological safety of food products (food fermentation) has as age-long practice (Campell-Platt and Adams, 1990). *Kunun-Zaki* is a Hausa word for a pleasantly fermented sweet-sour, cereal based, but non-alcoholic beverage. Although the production and consumption is predominant in Northern Nigeria, its acceptance and merchandise is fast spreading to the Southern parts of the country with perhaps about 60 – 80 million consumers. Gaffa and co-investigators (2002) reported that about 73% and 26% of people sampled in Bauchi and Gombe States of North-East Nigeria consume *Kunun-Zaki* daily and occasionally respectively. Millets (*Pennisetum typhoideum*), Guinea corn (*Sorghum bicolour*) and Maize (*Zee mays*) are the major cereals used in the production of the beverage in decreasing order of preference in Bauchi and Gombe States of Nigeria. The beverage is relished for its high refreshing attribute, energy boosting and for the fact that it is relatively cheaper than carbonated and other bottled beverages.

Although a number of researchers have embarked on various aspects of this beverage including its microbiology (Onuorah, *et al*. 1987; Egbere, 1988) nutritive value (Inatimi, *et al*. 1988) and improvements in its production (Gaffa and Ayo, 2002), the production technology of the beverage is still crude, unhygienic and requiring long hours of fermentation by chance microorganisms.

In this study, the researchers have attempted speeding up the fermentation process of *Kunun-Zaki* at the laboratory phase by re-introducing varying concentrations of a 24 hours old *Kunun-Zaki* (blackslopping) to the fresh sorghum starch paste and by varying the fermentation temperature with the view to determining possible optimal fermentation conditions.

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2.0 Materials and Methods

2.1 Sample Collection

Sorghum bicolor (grains), dry sweet potato chips (*Ipomoea batatas*) and dry ginger rhizome (*Zingiber officinale*) were purchased at Jos Main Market. Random samples of freshly prepared *Kunun-Zaki* were purchased from Bauchi Motor-Park, University of Jos' sale outlets, Main Market and 'Faringada' Markets of Jos Metropolis respectively.

2.2 Methods

a. Laboratory Production of *Kunun-Zaki*

The traditional method of *Kunun-Zaki* production as described by Onuorah (1987) and modified by Egbere (1988) were followed for the production of *Kunun-Zaki* at the laboratory, which when left for 24 hours served as a mother culture from which inoculum was derived. The flow chart for the production process is outlined in Figure 1.

b. Assessment of the Physico-chemical Quality Criteria Used for Determining Maturation Point of Fermentation of *Kunun-Zaki*.

Thirty samples of *Kunun-Zaki* randomly bought from Jos Metropolis were analyzed for total titratable acidity, pH and specific viscosity. The averages of the respective results of the parameters determined were used as standards for guiding the researchers on when to stop the fermentation process (that is, at the maturation point) at the laboratory. The pH values of the beverage samples were determined using a pH meter (pHep® 1 kaly Hanna Model), which has been previously standardized using buffer solutions of pH4 and 7 respectively. The total titratable acidity (TTA) calculated as lactic acid was determined following the method described by Pearson (1973). The specific viscosity of the samples was determined using specific Ostwald Fenke viscometer.

2.3 Studies on Optimized Fermentation Conditions of *Kunun-Zaki*

a. Effects of Temperature

To determine the effects of temperature alone on the fermentation rate, three replicates of 100ml each of sorghum starch as a fermenting slurry (with no inoculum) were prepared in 250ml conical flask and left at room temperature (28±2°C), 35°C and 45°C respectively to ferment while titratable acidity, TTA, and pH were determined on an hourly basis until maturation point was attained. The taste and aroma of the products were also determined for the matured products.

b. Effects of Inoculum's Concentration

Four conical flasks (250ml each) containing 70ml, 80ml, 90ml and 100ml of fresh starch (k*unu*) slurry were respectively inoculated with 30ml, 20ml, 10ml and zero ml (control) of inoculum respectively. The preparations were in triplicates and were left to ferment at a constant room temperature (28±2°C) while the pH and TTA were determined on hourly basis.

c. Combined Effect of Inoculum and Temperature

The combined effect of temperature and concentration of inoculum were determined by the use of 30% inoculum to ferment 'kunu' slurries set at room temperature (28±2°C), 35°C and 45°C respectively while parameters for assessing fermentation rate were determined on an hourly basis. At the end of each fermentation phase, other quality parameters – specific viscosity, taste and aroma were determined for the respective products.

d. Bacteriological Analysis

The total plate count (in colony forming units/ml) and identification of predominant microorganisms associated with the fermenting laboratory products were determined according to the methods described by Fawole and Oso (1988).

e. Sensory Evaluation

Differences in the taste, aroma and colour mouth feel general acceptability of the three sets of samples in the above experiments were carried out by a board of 10 taste panelists who were northerners that are acquainted with consumption of *Kunun-Zaki*. A five hedonic scale as described by Ihekoronye and Ngoddy (1985) was used to rank scoring of the products.

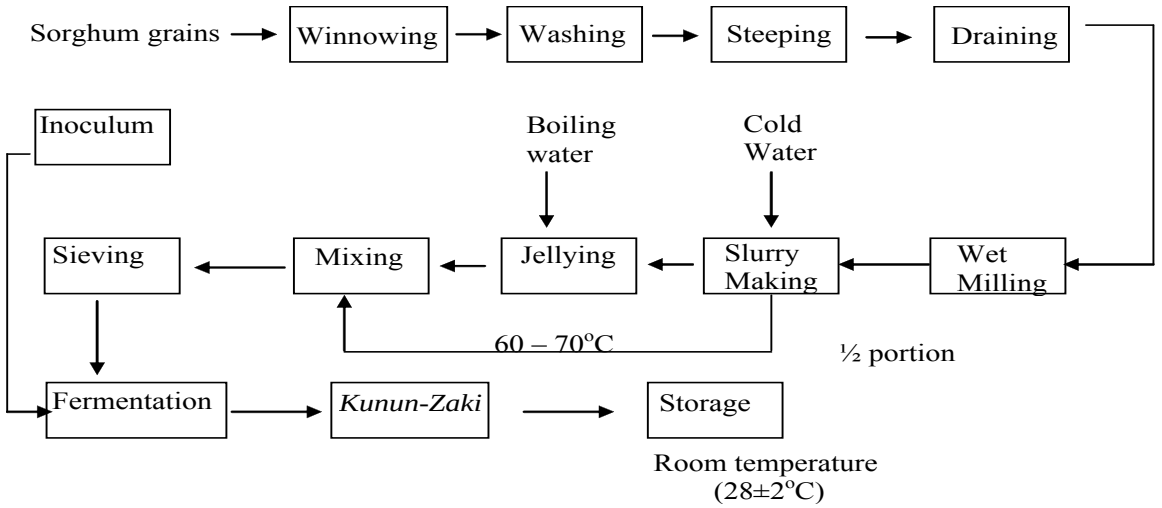

Figure 1: Procedure for the laboratory preparation of *Kunun-Zaki* (after Onuorah, 1987 and Egbere, 1988)

3.0 Results and Discussion

a. Physico-chemical Quality Indicators of Fresh *Kunun-Zaki*

The mean values of pH, total titratable acidity calculated as lactic acid, specific viscosity and specific density of the thirty randomly sampled *Kunun-Zaki* were 4.42±0.14, 0.030±0.003, 1.69±1.11 and 1.07±0.19 respectively. The three parameters (pH, acidity and specific viscosity) were used in adjudging the maturation point of fermentation in the course of the experiments.

The means of acidity values of the randomly sampled *Kunun-Zaki* in Jos metropolis were slightly higher than those obtained by Egbere (1988) in Makurdi, a state capital in Nigeria where room temperature was found to be equally higher (30±2°C) than the room temperature in Jos (28±2°C). This implies that ambient temperature could influence the degree of acidity – fermentation rate of traditionally produced *Kunun-Zaki*.

b. Effects of Temperature Alone on Fermentation Rate

The results in Table 1 show that fermentation rate increased with increase in temperature (inoculum concentratrion being constant) with the fastest rate obtained at 45°C. Fermentation rates at 35°C and room temperature respectively were found to be relatively slower. On the whole, 25% reduction in fermentation time was obtained by fermenting at 45°C without inoculum addition. Fermentation being an enzyme-dependent process (Penderson, 1971), and like all other enzyme-catalyzed reactions, increase in temperature would lead to a corresponding increase in fermentation rate. According to Blanchard (1992), higher temperatures could lead to increase in number of participating reactive molecules involved in bioreactiivity (fermentation). An advantage of fermenting *Kunun-Zaki* at 45°C is that most of the pathogenic mesophiles would be eradicated from the product, thereby making the product safer to consume.

c. The Effect of Inoculum Concentration Alone on Fermentation Rate

Inoculum addition to the fermenting '*kunu*' slurry (at constant temperature) led to a substantial reduction in fermentation time, i.e. increased fermentation rate (with increase in concentration of inoculum) by 30% resulting in a corresponding 41.67% decrease in fermentation time (from 12 to 7 hours) as shown in Figure 2. This is most likely due to the increased microbial population, which in-turn is a direct function of elaborated metabolites; in this case, acid(s) in the fermented product. The additional microbial load of the 24 hour–old *Kunun-Zaki* (see Table 2) must have contributed to the increased fermentation rate of the beverage.

d. Combined Effects of Inoculum Concentration and Temperature on Fermentation Rate

Variations in both inoculum concentration and experimental temperatures resulted in a reduction of fermentation time from 12 hours (at room tempera-

ture and zero inoculum concentration) to 4 hours at 45°C with 30% inoculum concentration (see Table 2). This resulted in a 66.67% (3 times) reduction in fermentation time. This also implies that the synergistic effects of both inoculum concentration and temperature elevation were more pronounced than the individual effects of temperature and inoculum concentration respectively.

e. Microorganisms Involved

It should be pointed out that the microflora of the fermented beverage samples shown in Table 2 is predominantly of those organisms indigenously associated with the raw sorghum grains and other raw materials used in the production of *Kunun-Zaki* (Egbere, 1988). The results also show that incubating the fermenting mixtures at the thermophillic temperatures of 45°C help to stratify the organisms into their temperature-loving zones. Only three bacterial species; *Lactobacillus*, *Streptococcus* and *Bacillus subtilis* were isolated from the fermenting mixture at 45°C, implying they are thermophillic, acid-producing and acid-tolerant organisms. The results also indicate that the organisms isolated from the fermented mixtures set at 35°C and at room temperature were all the same. This implies that they could have been mesophillic, acid-loving and some of them, acid-producers as well. Indeed fermentation at 45°C was found effective in eliminating *Corynebacterium sp*, *Enterobactercloacae* and *Staphylococcus aureus*.

f. Effects on Microbial Population

The initial microbial population (see Table 2) of the control samples without inoculum addition fermented at room temperature was found to be the lowest among the experimental samples. The obvious reason for this is the non-addition of the starter inoculum in the control fermenting mixture while the mixtures containing inocula had higher microbial populations, with the mixtures containing higher concentration on inocula having higher population densities.

g. Effects of Optimized Fermentation Condition on Sensory Quality

The results in Table 3 show that on a general note, the taste, odour and general acceptability of the product fermented at 45°C with an inoculum addition of 30% was more acceptable to the taste panelist than the other experimental and control samples (though, at an insignificant level, $P<0.05$). This implies that the increase in both temperatures cum inoculum concentration has a corresponding increase in the product acceptability. It invariably means that there was an increase in fermented metabolites (in this case, metabolized organic acids, aldehydes and other possible flavouring compounds with organoleptically – pleasing effects on the taste and aroma of the products.

The results on the ranked score quality parameters (see Table 3) however showed that there was no obvious alteration on the organoleptic quality of the beverage with respect to the optimized fermentation conditions ($P<0.05$).

Table 1: Effects of temperature variation on fermentation rate *Kunun-Zaki* (without Backslopping)

Time (Hours)	28±2°C		35°C		45°C	
	pH	TTA (%)	pH	TTA (%)	pH	TTA (%)
1.	6.52	0.005	6.52	0.005	6.52	0.005
2.	6.43	0.006	6.38	0.006	6.32	0.006
3.	6.31	0.006	6.20	0.016	6.05	0.018
4.	6.19	0.016	6.03	0.018	5.72	0.036
5.	6.01	0.019	5.83	0.037	5.38	0.038
6.	5.82	0.031	5.57	0.037	5.05	0.039
7.	5.61	0.037	5.35	0.039	4.68	0.040
8.	5.42	0.039	5.16	0.040	4.48	0.042
9.	5.23	0.039	5.01	0.041	4.26	0.045
10.	5.00	0.040	4.80	0.042		
11.	4.70	0.042	4.41	0.045		
12.	4.38	0.044	4.21	0.045		

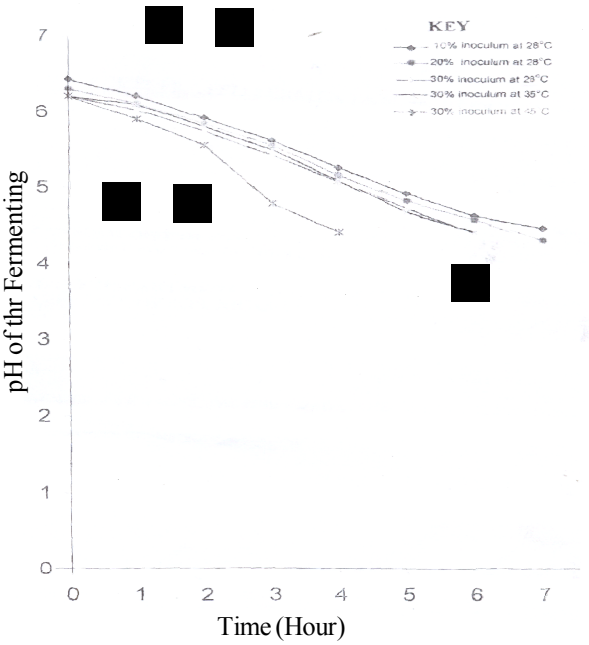

Figure 2: Effect of Inoculum Concentrations and Temperature on Fermentation Rate of *Kunun-Zaki*

Table 2: Effect of Backslopping and Temperature on the Microbial Population Density and Microflora of *Kunun-Zaki* during fermentation.

Sample Code	Fermentation Conditions and Duration: Temperature (°C)	Inoculum Cond. (%)	Fm Time (hours)	Reduction in Duration (%)	Microbial Load: Initial Load (cfu/ml)	Final Load (cfu/ml)	Increase (Log_{10})	Bacterial Isolates / Codes: K_1C	K_2E	K_3St_a	K_4L	K_5Str	K_6B_2
C2800	28±2	0	12	N/A (Control)	2.5×10^5	5.1×10^5	5.69	+	+	+	+	+	+
M28309	28±2	30	6	50.00	8.1×10^5	2.2×10^7	7.33	+	+	+	+	+	+
M3530	35	30	6	50.00	8.1×10^5	2.8×10^7	7.43	+	+	+	+	+	+
T4530	45	30	4	66.67	8.1×10^5	3.5×10^6	6.43	-	-	-	+	+	+

Key

K_1C	-	*Corynebacterium sp*	K_4L	-	*Lactobacillus sp*
K_2E	-	*Enterobacter cloacae*		K_5Str	-	*Streptococcus sp*
K_3St_a	-	*Staphylococcus aureus*		K_6B_s	-	*Bacillus subtilis*
+	-	Present		-	-	Absent
Fm	-	Fermentation		cfu/ml	-	Colony forming units per ml
C2800	-	*Kunun-Zaki* fermented at room temperature				
M3530	-	*Kunun-Zaki* fermented at 35°C with 30% inoculum addition				
T4530	-	*Kunun-Zaki* fermented at 45°C with 30% inoculum addition				
N/A	-	Not Applicable				

Table 3 *Sensory quality of Control and Experimental Samples of *Kunun-Zaki*

Kunun-Zaki Sample Codes	Colour	Odour	Taste	Mouth Feel	General Acceptability
C 2800 (Control)	3.50^a	3.20^b	3.30^c	3.00^d	3.20^e
M2830	3.30^a	3.40^b	3.40^c	3.20^d	3.20^e
M3530	3.20^a	3.40^b	3.60^c	3.60^d	3.50^e
T4530	3.30^a	4.00^b	3.80^c	3.80^d	4.10^e

Key

* Mean Scores with the same superscripts imply that there is no significant difference between them at 5% level of significance
M2830 - *Kunun-Zaki* fermented at room temperature with 30% inoculum addition.
C2800, M3530 and T4530 are as defined in Table 2.

4.0 Conclusion

The increase in fermentation rate with increase in incubation temperature as shown in this study is substantial by the explanation that fermentation being an enzyme-dependent process, when exposed to higher temperature could lead to increase in the numbers of participating reactive molecules involved in the bio-reaction process. The second advantage of fermenting *Kunun-Zaki* at 45°C besides the reduction in process time is the fact that most mesophillic pathogens could be eradicated (or reduced in population) from the beverage, thereby making the products relatively safer for consumption.

The increase in fermentation rate of *Kunun-Zaki* (with increase in inoculum concentration) could be due to increased microbial population, which in-turn is a direct function of the elaborated metabolites (in this case, acids). The study has also indicated that increasing both inoculum concentration and temperature could produce a synergistic effect in shortening fermentation time by far; up to 67%. It would therefore, be more economical to design a *Kunun-Zaki* fermentation vat that could operate at about 45°C, a temperature that is close to the optimum for industrial production of Yogurt (Adams and Moss, 1999), a similar acid-based fermented beverage.

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