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(RESEARCH ARTICLE)

Species of bacteria associated with laboratory and locally produced indigenous beverage, Kunun aya

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Abstract

A study was carried out on bacteria species associated with laboratory and locally-produced samples of Kunun aya. Dried *Cyperus esculentus* nuts were washed in three changes of sterile distilled water, further soaked in warm water for a period of 18 hours and blended into a fine paste. The paste was filtered with the aid of muslin cloth. The filtrate was simmered for a period of 15mins in order to concentrate the produce. Local spices were added to the resultant produce in order to improve on the taste. The produce was finally packaged into sachets and then refrigerated. Samples of locally produced Kunun aya was purchased from the market. Serial dilution was carried out on both laboratory and locally-produced samples of Kunun aya and incubated at 37^0C for the isolation of bacterial species. The products were also subjected to proximate analysis. Six bacterial species were isolated from the products. Least number of bacteria were isolated from the laboratory product as compared to the locally produced samples. The product contained essential nutrients that could also aid microbial growth. The ecological parameters recorded were within the ranges that could aid bacterial growth in pure culture. Refrigeration aids prolonged shelf-lives of the products. The use of non-contaminated water in the production of the product together with minimization of handling foods have been advocated to enhance the market value of the product.

Keywords: Kunun aya; Bacteria species; Local species; Temperature; Shelf-lives

1. Introduction

Nigeria abounds with diverse examples of locally produced beverages which could be classified into two groups namely; alcoholic and non-alcoholic beverages. Some alcoholic beverage includes 'palm-wine' produced from the sap of *Elaeis guinensis* and *Cocos nucifera* and coconut palm [1]; 'Burukutu' and 'Pito', which are fermented drinks produced mainly from the grains of cereals such as Sorghum [1], and 'Agadagidi', a fermented drink made from over-ripe plantains [2]. The non-alcoholic beverage includes 'Obiolor', a light brown beverage produced from malted sorghum or millet, or maize [3]; and 'Kunun zaki', a creamy beverage produced from millet grains. When compared to the industrially produced beverages, such as 'Coca-cola', 'Pepsi' and 'Five-Alive', these indigenous beverages are cheaper, and the ingredients used for their productions are locally sourced and available [4]. The local beverages are also consumed by people of low and middle income workers who cannot afford the conventional beverages [4]. The locally prepared beverages are usually consumed as after-meal drinks, or refreshing drinks in rural or urban centers in the company of friends or visitors, or in social gathering such as naming ceremonies, weddings and ordinations [5]. The other beverages that have gained worldwide acceptance which are non-indigenous to Nigeria include, soy milk, produced from soya bean (*Glycine max* – a legume) has been reported to be similar to cow milk in terms of protein and amino acid content [6]. A lot of researches have shown high level of contamination with the isolation of species of higher bacterial population, such as include; *Bacillus subtilis, Staplylococcus aureus, Escherichia coli, Streptococcus spp,* [7].

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2. Material and methods

The samples to be analyzed were collected aseptically, properly labeled and transported immediately to the laboratory for analysis. All media were prepared according to the manufacturers standards. Washed tigernuts were then soaked (steeped) in warm water (between 25-30 °C) for a period of 10 - 18 hours. The tigernut were then put into 400 ml of sterile distilled water and blended with a kitchen blender with some local spices (Masoro, Kananpari, and Chitta) to a fine paste as described by Abaejoh *et al.* [8]. The paste was sieved or filtered using a piece of muslin cloth. Pressure was applied to the content to achieve a maximum liquid extraction. The filtrate was then simmered for 10-15 minutes in order to concentrate the resultant tiger nut extract and to give the milk a form of pasteurization. The addition of local spices was to give the extract a pleasant taste and aids its preservation. It was allowed to cool, packaged in a sealed sachet and then refrigerated in order to aid a longer shelf life.

The volume of the final yield was recorded and subsequently dispensed into 50 cl plastic bottles. A volume of 5 ml of the laboratory prepared sample and the samples from sales outlets were analyzed for moisture content, ash, dry matter, crude protein, crude fiber and ether extract (fat) using Standard methods described by [9]. Sample from the laboratory prepared Kunun aya was subjected to organoleptic assessment based on colour, taste, flavour, consistency, and overall acceptability using 10-member semi-trained panelists.

Isolation of microorganisms associated with Kunun aya drink was carried out using standard method described by [10]. These Standard procedures of identification of microbial isolates described was based on classification scheme proposed by [10].

The laboratory-produced samples were aseptically packaged in 50 cl bottles and stored under the following conditions, room temperature (25-30 ^{o}C) for a period of 21 hour per day, in a 0–21 hours, Cooler-with Ice block (chilling) temperature (12-30 ^{o}C) for a period of 28 hours daily, and in a refrigeration temperature (10±2 ^{o}C) for a period of 63 hours daily and for a total period of three days. The data were subjected to a two-way factors Analysis of Variance (ANOVA).

3. Results

3.1. Proximate analysis of beverage

All samples had high values of moisture, crude protein, crude fiber, dry matter, relatively low values of fat and ash contents. High values in protein content was observed on all samples which implies high nutrition content. Samples showed high percentage of fiber content, implying high energy constituent of kunun aya making it an energetic beverage drink. High content of ash in the three samples analyzed, implied high percentage of mineral content of kunun aya. Natural spices added to this drink acted as flavouring agents and preservatives to some extent which could have antimicrobial effects.

Table 1 Proximate analysis of beverage

Sample	Analysis in percentage (%)					
	DM	Moisture	Ash	CP	EE (FAT)	CF
A	11.40	89.50	3.50	13.10	4.00	7.00
B	11.25	88.75	4.16	12.89	3.51	7.49
C	11.24	82.76	4.11	13.49	3.49	7.52

DM= Dry Matter, CP= Crude Protein, EE= Ether Extract (fat), CF= Crude Fibre.

3.2. Organoleptic Evaluation of Beverage

The mean organoleptic evaluation scores obtained from the panel evaluation of the samples may stem from the time of production (0 – 24 hrs). There was no significant different in general acceptance of all samples analysed, although the laboratory-produced sample had the highest acceptance in terms of taste, aroma, colour. The aroma from 0 – 48-hour beverage of the laboratory-produced sample was highly and significantly preferred over the two samples from Yelwa market and Yelwa campus.

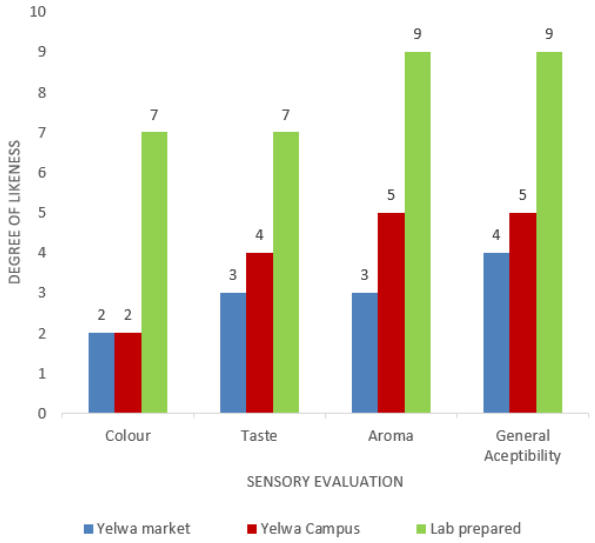

Figure 1 Organoleptic evaluation of tigernut extract

3.3. Frequency of occurrence of bacterial isolates from the beverage

Bacterial isolates obtained from samples A, B, and C (Yelwa market, Yelwa campus, and laboratory-produced sample, respectively) revealed high counts of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Streptococcus sp, Salmonella sp* and *Shigella sp,* High numbers were isolated from sample A and B. Few counts were obtained from sample C (laboratory-produce).

The results of the findings indicate high bacterial counts, which could be as a result of using contaminated water or failure to adhere to personal hygiene during production of the drinks. Under the tropical climate characterized by ambient temperature above 30^0C, rapid increase of microorganism is likely to occur in the beverage [11].

Table 2 Frequency of occurrence of bacterial isolates in the samples

Bacterial Isolates	A	B	C	Total	Incidence (%)
Staphylococcus aureus	+	+	+	3	27.27
Escherichia coli	+	+	+	3	27.27
Bacillus subtilis	+	+	-	2	18.18
Streptococcus sp	-	+	-	1	9.09
Salmonella sp	+	+	+	1	9.09
Shigella sp	+	-	-	1	9.10
Total	4	5	2	11	100

A= Yelwa market sample, B= Yelwa campus sample, C= Laboratory-produced sample
+ = Present and - = Absent

3.4. Determination of bacterial counts

Sample from Yelwa campus (A) had the highest counts in all the dilution factors, except at 10^{-3} where sample B recorded higher bacterial counts, sample C had the least growth, all the counts did not exceed 100cfu/ml. for each treatment. The bacterial growth from samples A and B cultured on MacConkey Agar suggest that retailed samples could be contaminated with Coliforms. Sample C (Laboratory—produce) recorded no growth on MacConkey agar, this suggests that good manufacturing practice was adhered during production. ANOVA was used for analyses which showed no variation among the TPC in the three sites at $P>0.001$.

The total plate count recorded was 1.97x10^5 cfu/ml sample A, 1.87x10^5 cfu/ml sample B, and 4.20x104 cfu/ml sample C which had the highest count. The total viable counts recorded in this study is similar to those reported by Onovo and Ogaraku (2007) [7] and Musa and Hamza (2013) [12].

A total coliform count of sample A and B ranged from 1.48x10^5 - 2.10x10^5 cfu/ml, with sample B having the highest count. No Coliform was in the laboratory prepared sample. The non-detection of *Coliforms* in laboratory-prepared sample C could have resulted from good manufacturing and hygiene practices observed during production. *Coliforms* are of fecal origin and their presence in food indicates contamination from fecal sources which is highly undesirable. *Coliforms* such as *Escherichia coli* can cause diseases such as gastroenteritis, diarrhea, and urinary tract infection. These reports also correspond to the findings of Ayo et al., (2013) [11].

Salmonella/Shigella Counts, values of 1.08x10^5 - 1.96x10^{5}cfu/ml were recorded for samples A and B respectively with sample B having the highest counts. There was no growth on the laboratory prepared sample C. This could be due to heat treatment (pasteurization) given to the beverage during production [7] [13] [12].

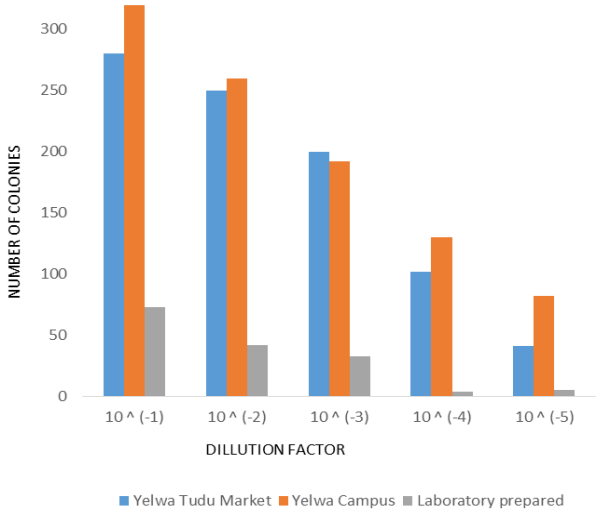

Figure 2 Total aerobic bacterial count on nutrient agar

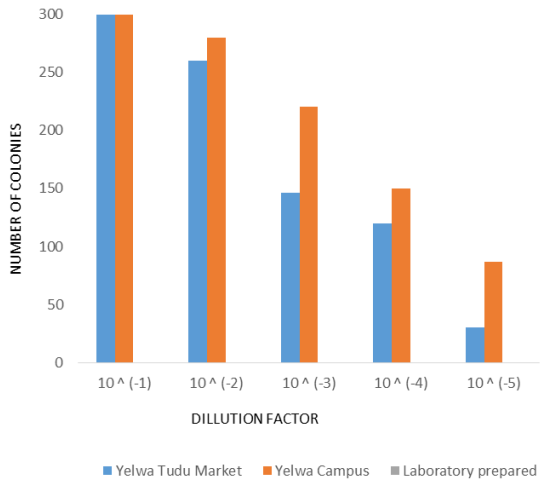

Figure 3 Total aerobic bacterial count on Macconkey agar

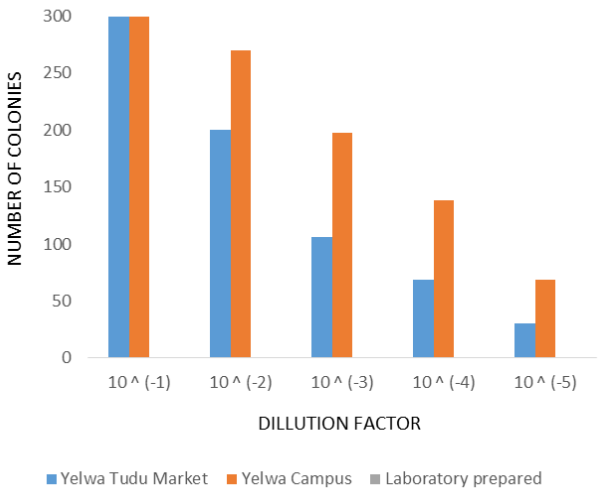

Figure 4 Total aerobic bacterial count on Salmonella/Shigella agar

3.5. Effects of temperatures on shelf-life of the product

There was no significant bacterial growth for a period of 15- 21 hours after the production and storage of the beverage ($3.9x10^{-2}$, $2.9x10^{-2}$, and $2.0x10^{-2}$) at room, chilling and refrigerating temperatures respectively.

Table 3 Keeping quality of the product at room temperature

Time (hour)	Appearance	TBC (cfu/ml)	Inference	Isolates identified
0 -7	Milky-white, Fresh and sweet	NG	Very good	Nil
8 -14	Fresh and sweet	NG	Very good	Nil
15 -21	Milky-white turns brown	$3.9x10^{-2}$	Deterioration begins	*S. aureus, E. coli*
22 -28	Brownish in colour	$4.5x10^{-2}$	Deteriorated	*S. aureus, E. coli*
29 -35	Brownish and clumsy	$6.5x10^{-2}$	Deteriorated	*S. aureus, E. coli*
36 – 42	Clumsy and sticky	$7.3x10^{-2}$	Deteriorated	*S. aureus, E. coli, Str sp.*
43 – 49	Clumsy and sticky	$9.7x10^{-2}$	Deteriorated	*S. aureus, E. coli*
50 – 56	Bad odour	$1.05x10^{-3}$	Deteriorated	*S. aureus, E. coli*
57 -63	Bad odour	$1.42x10^{-3}$	Deteriorated/ discarded	*S. aureus, E. coli*

NG: No growth, TBC: Total bacterial counts, *Str. Sp.*: *Streptococcus spp*

Table 4 Effects on shelf life of product at chilling temperature

Time (hour)	Appearance	TBC (cfu/ml)	Inference	Isolates identified
0 -7	Milky-white, Fresh and sweet	NG	Very good	Nill
8 -14	Fresh and sweet	NG	Very good	Nill
15 -21	Sweet	$2.9x10^{-2}$	Good	*S. aureus, E. coli*
22 -28	Milky white turns brown	$3.8x10^{-2}$	Deteriorated begins	*S.aureus, E. coli*
29 -35	Brownish colour	$4.3x10^{-2}$	Deteriorated	*S. aureus, E. coli*
36 – 42	Clumsy-appearance	$5.5x10^{-2}$	Deteriorated	*S. aureus, E. coli, Str sp.*
43 – 49	Clumsyand sticky	$6.2x10^{-2}$	Deteriorated	*S. aureus, E. coli, Str sp.*
50 – 56	Bad odour	$8.8x10^{-2}$	Deteriorated	*S. aureus, E. coli, Str sp.*
57 -63	Bad odour	$1.00x10^{-2}$	Deteriorated/discarded	*S. aureus, E. coli, Str sp.*

NG: No Growth, TBC: Total Bacterial Counts, *Str. Sp.*: *Streptococcus spp*

There was a change in the appearance at room temperature, but no change was observed at chilling and refrigerating temperatures, the beverage taste was still good. An increase in storage showed gradual change in the appearance and taste of the stored beverage as well as an increase in the number of colonies ($1.42x10^{-3}$, 1.00×10^{-3}, and $8.7x10^{-2}$) at room, chilling and refrigerating temperatures respectively, and products after 57 – 63 hours decayed and was therefore discarded. *Staphylococcus aureus* and *Escherichia coli* were isolated from the room temperature stored beverage after 15 hours.

Table 5 Effect s of refrigeration temperature on shelf-life of product

Time (hour)	Appearance	TBC (cfu/ml)	Inference	Isolates identified
0 -7	Milky white, Fresh and sweet	NG	Very good	Nill
8 -14	Fresh and sweet	NG	Very good	Nill
15 -21	Sweet	$2.0x10^{-2}$	Good	*S. aureus, E. coli*
22 -28	Sweet	$2.7x10^{-2}$	Good	*S. aureus, E. coli*
29 -35	Sweet	$3.3x10^{-2}$	Good	*S. aureus, E. coli*
36 – 42	Sweet	$3.9x10^{-2}$	Good	*S. aureus, E. coli*
43 – 49	Milky white turns brown	$5.9x10^{-2}$	Deteriorated begins	*S. aureus, E. coli*
50 – 56	Brownish/unpleasant odour	$7.8x10^{-2}$	Deteriorated	*S. aureus, E. coli*
57 -63	Bad odour	$8.7x10^{-2}$	Deteriorated /discarded	*S. aureus, E. coli*

NG: No Growth, TBC: Total Bacterial Counts.

4. Conclusion

The bacterial contaminants of the market samples may have originated from the source of water employed for the beverage production or through handling faults. The prevailing tropical temperature must have favoured the rapid multiplication and flourishing of the bacterial isolates. The beverage was also found to contain the essential food nutrients which could have also favoured the growth and survival of the bacterial isolates. The presence of Coliform in the beverage as determined in this study could be of public health concern because of teaming populace, especially students that consumes this beverage as an alternative to bottled soft drinks. The presence bacteria isolates may also pose a special health risk on human beings especially the infants, young children, pregnant women and people with severely compromised immune system. The presence of *Staphylococcus aureus,* could be as a result of handling faults by the producers because these organisms belong to flora of the skin, which may have possibly found its way by manual contamination. *Bacillus subtilis* may have also found its way into the beverage through the soil, water and failure to adhere to simple safe production practices. *Salmonella sp* and *Shigella sp* may have colonized the beverages through handling faults. However, good manufacturing and good hygiene practices should be given utmost importance during production to avoid microbial contamination that may cause food-borne illness.

Compliance with ethical standards

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Disclosure of conflict of interest

There's no what so ever any conflict of interest we are giving the outfit full right to publication of this work.

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