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SENSORY AND MICROBIAL EVALUATION OF PEARL MILLET KUNUN ZAKI SACCHARIFIED WITH MALT FROM SOME PEARL MILLET AND SORGHUM CULTIVARS

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ABSTRACT

Kunun zaki formulations were produced with pearl millet cultivar (SOSAT C-88), ginger (Zingiber officinale, black pepper (Piper quineense), hot pepper (Capsicum frustescens) and 5% malt from six pearl millet cultivars (SOSAT C-88, ZANGO, EX-BORNO, ICMV - IS 94206, GWAGWA, GB 8735) and one sorghum cultivar (ICSV III) were added to produce seven formulations, in addition to the control sample without malt. Microbiological quality of eight kunun zaki formulations was evaluated. Twenty six panelists were used to determine the acceptability of kunun zaki from the eight kunun zaki formulations using a nine-point Hedonic scale. Addition of 5% malt did not affect the microbial count of kunun zaki formulations. Saccharomyces cerevisiae, Klebsiella aerogenes and Aspergillus niger were the microorganisms isolated from all the kunun zaki formulations. Shigella and Salmonella were not encountered in this study. Addition of 5% SOSAT C-88, ZANGO, EX-BORNO, ICMV-IS 94206 and GWAGWA malt improved the taste and texture of kunun zaki formulations. The population of the microorganisms isolated from kunun zaki formulations was not high enough to produce effective dose. However, the need for processors of kunun zaki to adopt strict hygiene practices cannot be over emphasized.

Key Words: Kunun zaki, saccharification, pearl millet, malt, microbiology, sensory evaluation

INTRODUCTION

Kunun zaki is a popular non alcoholic cereal beverage consumed widely in Nigeria, particularly in the semi arid region during ceremonies and also when the weather is hot with snacks and home use. There are several reports on *kunun zaki* processing using sweet potatoes (Osuntogun and Aboaba, 2004), malted rice (Akoma *et al.*, 2002), malted sorghum (Ariahu *et al.*, 2005) and *Cadaba farinose* (Gaffa and Ayo, 2003) as saccharifying agents.

The investigations carried out have been based on the production, acceptability and microbiological quality of *kunun zaki* using sweet potatoes, malted rice, malted sorghum and *Cadaba farinosa* as saccharifying agents other than malted pearl millet. Pearl millet grain contains high level of amylase activity and incidentally is the common

cereal grain in the semi-arid parts of Nigeria. This prompts the need for the investigation into the liquefying and saccharifying ability of pearl millet cultivars and its malt in *kunun zaki* preparation. Also there is the need to produce the beverage with pearl millet grain and determine the effect of this grain and its malt on the acceptability and microbiological quality of the final beverage. The objective of this study was to produce *kunun zaki* with pearl millet grain, saccharified with malt from various pearl millet cultivars and sorghum, and determine the acceptability and microbiological quality of the beverages.

MATERIALS AND METHODS

Six pearl millet cultivars; SOSAT C-88, ZANGO, EX-BORNO, ICMV–IS 94206, GWAGWA, GB 8735 and Sorghum (ICSV III) were obtained from Lake Chad Research Institute, Maiduguri and International Crops Research Institute for the Semi-Arid Tropics Experimental Station at Bagauda, Kano, Nigeria. These pearl millet cultivars were used because they have negligible tannin content (Badau *et al.*, 2002), high hydrochloric acid extractability of minerals (Badau *et al.*, 2005 a), high sugar content (Badau *et al.*, 2005 b), high amylase activities (Badau *et al.*, 2006 a) and generally, they have good malting properties (Badau *et al.*, 2006 b). Culture media, chemicals and reagents for biochemical tests were obtained through recognized distributors in Nigeria and were of analytical grade.

Preparation of *kunun zaki* ingredients

The spices (ginger, black pepper and hot pepper) were cleaned thoroughly and milled with hammer mill (Gibbons Electric, Essex, U.K.) to pass through 1 mm mesh size screen. About 8 Kg of pearl millet cultivar (SOSAT C-88) was cleaned and soaked for 12 h. The soak water was changed for every 6 h of soaking. The soaked grain was milled with attrition mill (Yamaha model, Japan) into paste.

Malting

The pearl millet cultivars were malted as described by Badau *et al.* (2005 a). The grains were steeped at room temperature (32 ± 2^0C) for 12 h. The steep liquor was changed after 6 h. One air rest period of 1 h was applied after 6 h of steeping. After steeping, the grains were washed and drained. The grains were immersed in a 0.1% (vol/vol) solution of a commercial bleach (parazone) containing 3.5% sodium hypochlorite (diluted to 0.1% by taking 5 ml of parazone and made up to 175 ml with distilled water) for 20 min as reported by Morall *et al.* (1986). After sterilization, the grain was wrapped in wet pieces of cotton cloth and placed on wet jute bag. Another wet jute bag was used to cover the grains wrapped in the wet cotton cloth (Obizoba and Atii, 1994). The pearl millet grains were allowed to germinate at room temperature (32 ± 2^0C) for 72 h. During germination, small quantity of water (15 ml) was sprayed on the germinating grains using an atomizer spray and they were "turned" (by moving a clean wooden rod inside the germinating grains) at the same time (Aniche, 1989). At the end of germination, the germinated grains were dried to moisture content of 5.00 ± 0.5% in a fan fitted oven (Gallenkamp, England) for 24 h. The dried germinated grains were polished by removing roots and rootlets. Rootlets and shoots of the grains were separated from kernels by rubbing between the palms in a sieve (Endecotts Ltd, London, England) of 0.6 mm mesh size. The sieve allowed the rootlets and shoots to escape but retained the kernels (Morall *et al.*, 1986; Aniche and Palmer, 1990). The polished malt was milled into flour with hammer mill (Gibbon Electric, Essex, U. K.) to pass through 1 mm mesh screen, packed in plastic containers and stored in iron cupboard shortly before use.

Kunun zaki formulation

The recipe used for the *kunun zaki* formulations is given in Table 1. *Kunun zaki*

was prepared as described by Gaffa and Jideani (2001) with little modification. The paste of SOSAT C-88 and sweet potato was divided into two portions (2/3 and 1/3). One litre of hot water (100 °C) was added to the 2/3 portion and was allowed to cool to 70 ±5°C. The 1/3 portion along with the milled spices and malt flour was added to the paste at 70 ± 5°C, mixed thoroughly and allowed to saccharify for 6 h. After 6 h, it was filtered and sugar added to taste. Malt flour from the 6 pearl millet cultivars and one sorghum cultivar were used to produce 7 formulations (with malt) and one formulation without malt (control), making a total of 8 formulations. The procedure is shown in Figure 1 and formulations of the various *kunun zaki* are also shown in Table 1.

Table 1. Recipe for pearl millet *kunun zaki* formulations

Ingredient	Formulations							
	I	II	III	IV	V	VI	VII	VIII
Pearl millet (g)	1000	1000	1000	1000	1000	1000	1000	1000
Sweet potatoes (g)	600	600	600	600	600	600	600	600
Ginger (g)	5	5	5	5	5	5	5	5
Black pepper (g)	2	2	2	2	2	2	2	2
Hot pepper (g)	1	1	1	1	1	1	1	1
Pearl malt flour (g)								
SOSAT C-88	-	50	-	-	-	-	-	-
ZANGO	-	-	50	-	-	-	-	-
EX-BORNO	-	-	-	50	-	-	-	-
ICMV-IS 94206	-	-	-	-	50	-	-	-
GWAGWA	-	-	-	-	-	50	-	-
GB 8735	-	-	-	-	-	-	50	-
Sorghum malt flour (g)								
ICSV III	-	-	-	-	-	-	-	50
Water (ml)	3500	3500	3500	3500	3500	3500	3500	3500
Sugar (g)	100	100	100	100	100	100	100	100

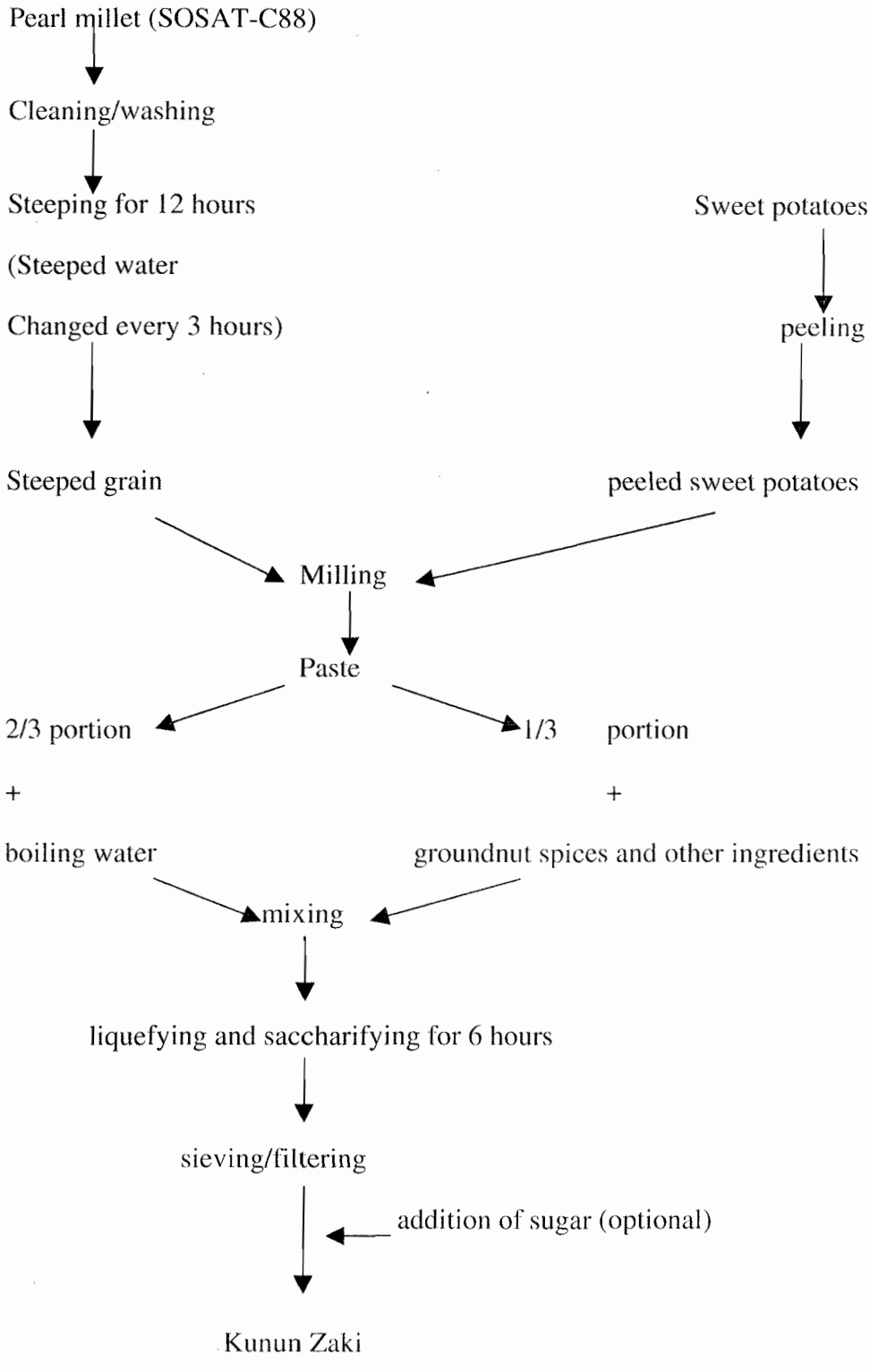

Figure 1. Flour diagram for pearl millet *kunun zaki* production.
Source: Gaffa and Jideani (2001).

Sensory evaluation for *kunun zaki*

Kunun zaki was evaluated by 26 panelists who were familiar with the beverage. They were asked to take a sip and rank the beverage on the basis of taste, texture, colour, aroma and overall acceptability using a nine-point Hedonic scale (Larmond, 1977).

Kunun zaki formulations were served to the panelists in white and transparent glass cups and were asked to rinse their mouth with fresh water at room temperature, before next serving. The containers with the samples were coded and kept far apart to avoid crowding and for independent judgement. Although, the panelists were not trained but their selection was based on basic requirements of a panelist, such as availability for the entire period of evaluation, interest, willingness to serve, good health (not suffering from colds), not allergic or sensitive to the products evaluated (Penfield and Campbell, 1990).

Microbiological analysis of *kunun zaki*

The total bacterial, coliform, yeast and mould counts of pearl millet *kunun zaki* formulations were carried out as described by Collins and Lyne (1970), Harrigan and McCance (1976).

Preparation of samples for microbial studies

Samples of 8 kunun zaki formulations were placed in sterile sampling containers and kept in refrigerator prior to microbiological analysis.

Preparation of Culture Media

Culture media for mould count were prepared in accordance with the methods described by Collins and Lyne (1970), Harrigan and McCance (1976). The culture media prepared were nutrient agar (International Diagnostics Group Plc, Plancashire BC9 6AU, U.K) for total bacterial count, mannitol salt (International diagnostic group PLC, U.K.) for staphylococcal count, sabouraud dextrose agar (Biotech Laboratories, 36 Ansuon Road) for yeast count, salmonella/shigella agar (International Diagnostic group Plc, U.K) for *Salmonella/Shigella* detection and potatoes dextrose agar (BDH Chemicals L-Poole, England) for mould count. All microbial colonies were subcultured on their respective culture medium to obtain pure cultures.

Isolation and Identification of bacteria, moulds and yeasts

Kunun zaki formulations were serially diluted up to 10^{-6} dilution. Each diluent of the samples was plated out in duplicate using the pour plating technique as described by Harrigan and McCance (1976). The plates were incubated aerobically for 24 to 48 h at 37°C for total viable counts, 48 h at 37°C for coliform count, 48 h at 37°C for staphylococcal count (Badau *et al.*, 1999) and 72 to 96 h at room temperature (32 ± 2°C) for fungal counts. After incubation period for bacteria and fungi, their colonies appearing on the agar plates were counted using a digital colony counter. The average colony obtained from the countable duplicate plates, were expressed as either colony forming unit per gram (cfu/g) or colony forming unit per milliliter (cfu/ml) of sample. All pure cultures of bacteria, moulds and yeast were maintained on nutrient agar, potatoes dextrose agar and sabouraud dextrose agar slants, respectively. These isolates were kept in the refrigerator prior to identification.

The cultural characteristics of discrete colonies such as colour, shape and pigmentation of the colonies were observed and noted. It was followed by characterization of the isolates using microscopic examination for cell morphology. Microscopic examinations for detailed cell morphology and Gram reaction were carried out on 24 h old

bacteria cultures (Harrigan and McCance, 1976). Biochemical tests were conducted on each isolate for the tests. The isolate was inoculated in peptone water and left for 24 h at 37°C. Biochemical tests carried out on all the bacteria isolates in each sample were starch hydrolysis, catalase test and utilization of sugars. The sugars used were glucose, sucrose, mannose, mannitol, xylose, arabinose, lactose, galactose, starch, maltose and fructose. Voges Proskauer, nitrate reduction, indole and motility tests were also carried out. These tests were carried out using the methods described by Collins and Lyne (1970); Harrigan and McCance (1976). Results obtained from these tests were compared with literature standards using diagnostic tables showing the biochemical reactions identifying many genera and species of bacteria (Cowan and Steel, 1961).

Fungal isolates were identified based on cultural and morphological characteristics. Slide culture for identification based on morphological characteristics was prepared by taking an inoculum from the edge of an isolated colony, placing it on a slide and mounting fluid (Lactophenol cotton blue) added. This was covered with a cover slip and heated on a flame to expel air bubbles. The prepared slide was mounted on a microscope and the morphological characteristics and types of spores noted. The moulds were identified as reported by Gilman (1957), Gaffa and Jideani (2001), and Singh *et al.* (1991). The percentage frequency of microorganisms was calculated based on percentage occurrence on plated samples (Badau *et al.*, 1999).

Statistical analysis

Statistical analysis was carried out as described by Mead *et al.* (1993) and with computer programme, statistix, version 4.1 U.S.A. Analysis of variance (ANOVA) and student t- test were used to determine the differences among and within sensory scores and microbial counts of *kunun zaki* formulations.

RESULTS AND DISCUSSION

Sensory quality of *kunun zaki*

The quality of *kunun zaki* from pearl millet cultivar (SOSAT-C88) as affected by addition of malt from various grains is shown in Table 2. The addition of malt at 5% level of the weight of the grain used for the *kunun zaki* preparation did not significantly ($P<0.05$) affect the aroma, colour and overall acceptability of *kunun zaki*. However, the taste and texture of the various *kunun zaki* were affected by the addition of malt. The taste of pearl millet kunun zaki with added SOSAT C-88 malt, ZANGO and SOSAT IS94206 were

Table 2. The quality of *kunun zaki* from pearl millet cultivar (SOSAT - C88) as affected by malt from various pearl millet cultivars and sorghum[1]

	Sensory Scores				
Weaning Food[2]	Aroma	Colour	Taste	Texture	Overall Acceptability
Formulation I	6.12^{a}	6.77^{a}	5.92^{b}	6.3^{c}	6.15^{a}
Formulation II	7.27^{a}	7.15^{a}	6.77^{a}	7.27^{a}	7.19^{a}
Formulation III	6.96^{a}	7.00^{a}	7.00^{a}	7.39^{a}	7.08^{a}
Formulation IV	6.73^{a}	7.23^{a}	6.54^{ab}	7.23^{ab}	6.92^{a}
Formulation V	7.08^{a}	6.77^{a}	6.85^{a}	7.23^{ab}	7.00^{a}
Formulation VI	6.62^{a}	6.92^{a}	6.31^{ab}	6.85^{abc}	6.65^{a}
Formulation VII	6.50^{a}	6.89^{a}	5.92^{b}	6.50^{bc}	6.54^{a}
Formulation VIII	6.69^{a}	7.15^{a}	6.23^{ab}	6.46^{c}	6.27^{a}

[1]Mean of triplicate determinations

[abc]Means within each column not followed by the same superscripts are significant ($P<0.05$) different

[2]Formulations I = no malt, II = 5% SOSAT C – 88 malt, III = ZANGO, IV = EX-BORNO, V = ICMV –IS 94206, VI = GWAGWA, VII = GB 6735 and VIII = Sorghum

preferred (P<0.05). On the other hand, the texture of pearl millet *kunun zaki* with added malt from ICMV-IS ZANGO, SOSAT-C88, EX-BORNO and 94206 were most preferred (P<0.05). Generally, pearl millet *kunun zaki* that had malt were most preferred (P<0.05). This could not be unconnected with the activity of malt which is a package of enzymes (Hough, 1991). The action of the various enzymes on the starchy structure of the grains used for *kunun zaki* preparation could have increased the sweetness and smoothness of the *kunun zaki* beverage.

Microbiology of *kunun zaki*

The microbial count $\log_{10}$ (cfu/g) of *kunun zaki* from various formulations showed that the total bacterial count ranged from 4.42 to 4.62 $\log_{10}$ (cfu/g), coliform 4.16 to 4.67, yeast 3.51 to 4.34, staphylococcal 3.12 to 3.64 and mould from 1.26 to 2.14 (Figure 2). Formulations did not affect the microbial count of the *kunun zaki* formulations to a large extent.

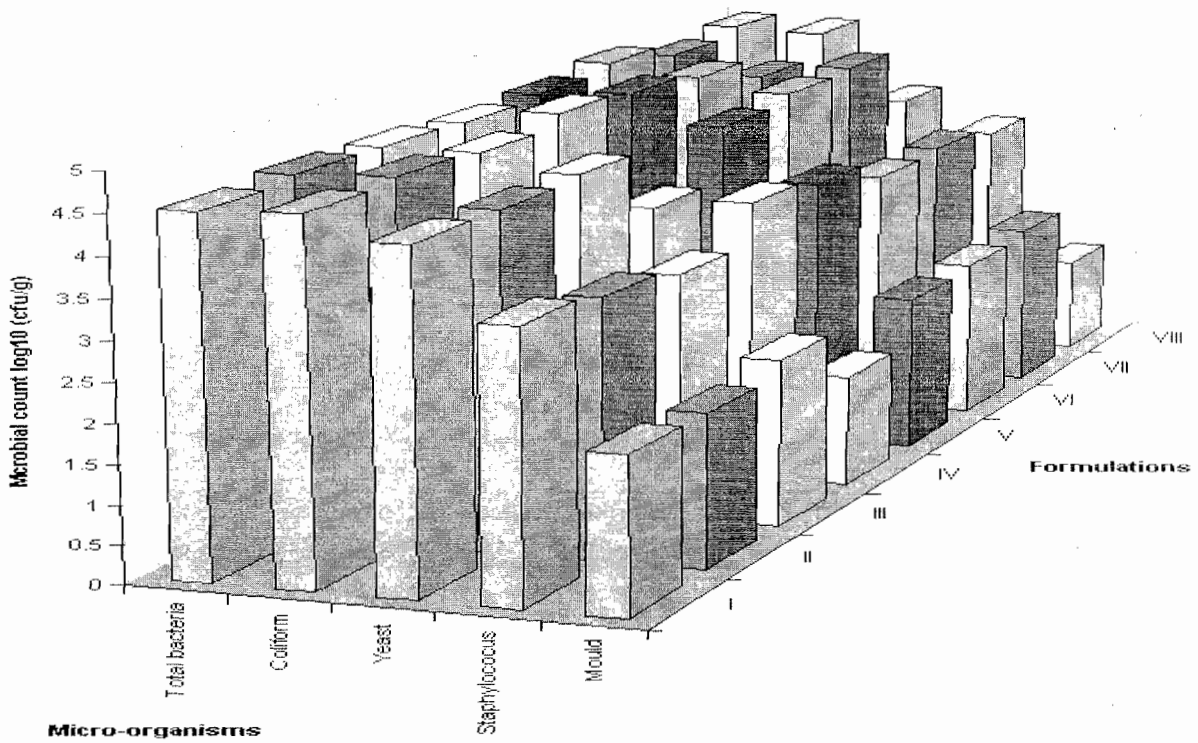

Figure 2. Microbial count of kunun zaki formulations

Formulations I = no malt, II = 5% SOSAT C-88 malt, III =ZANGO, IV = EX-BORNO, V = ICMV-IS 94206, VI = GWAGWA, VII = GB 8735 and VIII = Sorghum

Table 3. Distribution of microorganisms and their percentage frequency of occurrence in *kunun zaki* formulations[1]

	Kunun zaki Formulations[2]							
Microorganism	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Candida tropicalis	16.67	-	-	45.83	75.00*	70.83	-	70.83*
Saccharomyces cerevisiae	33.33	33.33	45.83	37.50	54.17	70.83	75.00*	70.83*
Candida utilis	29.17	-	33.33	-	-	70.83	-	-
Staphylococcus aureus	12.5	58.3*	41.7	37.5	16.7	37.5	25.0	33.3
Streptococcus lactis	33.33	-	66.67*	50.00	41.67	50.00	62.50	45.83
Lactobacillus bulgaricus	41.67	12.50	-	20.83	-	45.83	-	-
Leuconostoc mesenteroides	45.8.	45.83	-	33.33	45.83	-	-	-
Bacillus subtilis	54.17	-	-	45.83	62.50	54.17	62.50	62.50
Proteus vulgaris	62.50*	-	-	-	-	45.83	66.67	58.33
Klebsiella aerogenes	20.83	12.50	20.83	12.50	8.33	16.67	12.50	25.00
Candida albicans	-	41.67	50.00	-	-	-	75.00*	-
Candida pseudotropicalis	-	45.83	45.83	62.50*	-	-	75.00*	-
Lactobacillus plantarum	-	20.83	-	-	-	-	-	-
Escherichia coli	-	8.33	16.67	20.83	8.33	12.50	-	20.83
Micrococcus varians	-	-	12.50	-	12.50	-	-	-
Protens vulgaris	-	-	62.50	-	-	-	-	-
Lactobacillus delbruekii	-	-	-	-	-	-	58.33	-
Bacillus coagulans	-	-	54.17	-	-	-	-	54.17
Aspergillus niger	12.30	25.00	20.83	33.33	20.83	20.83	4.17	12.50
Rhizopus arrhizus	33.33	-	45.83	54.17	-	75.00*	-	45.83
Aspergillus fumigatus	20.83	12.50	25.00	37.50	45.83	25.00	20.83	-
Aspergillus nidulans	-	25.00	-	-	25.00	-	33.33	25.00

- = nil

[1]Frequency calculation was based on percentage occurrence in plated materials

[2]Formulations I = no malt, II = 5% SOSAT C – 88 malt, III = ZANGO, IV = EX-BORNO, V = ICMV –IS 94206, VI = GWAGWA, VII = GB 6735 and VIII = Sorghum

*Most occurring/predominant microorganisms

The distribution of microorganisms and their percentage frequency of occurrence in *kunun zaki* formulations are shown in Table 3. The most frequent microorganism that occurred in formulation I (62.5%) was *Proteus vulgaris*. On the other hand, *Staphylococcus aureus was the* predominant microorganism in *kunun zaki* formulation II (58.3%). *Streptococcus lactis, Candida pseudotropicalis, Candida tropicalis* and *Rhizopus arrhizus* occurred most frequently in formulations III (66.67%), IV (62.50%), V (75.00%) and VI (75.00%), respectively. *Saccharomyces cerevisiae, Candida albicans* and *Candida pseudotropicalis* were the predominant microorganisms in formulationVII at relatively higher frequency (75.00%). *Candida tropicalis* and *Saccharomyces cerevisiae* are the microorganisms that occurred more frequently in *Kunun zaki* formulation VIII (70.83%). *Staphylococcus aureus, Klebsiella aerogenes* and *Aspergillus niger* were widely distributed. It occurred in all the pearl millet *kunun zaki* formulations. Microorganisms encountered in this study have been isolated by Gaffa *et al.* (2002) in *kunun zaki*. Although one species of coliform bacteria was isolated, *Salmonella sp* was not detected. The microorganisms isolated were mostly spoilage bacteria. Gram positive spore forming rods isolated from *kunun zaki* were *Bacillus subtilis* and *Bacillus coogulans*, while the Gram positive none spore forming rods were *Lactobacillus plantarum, Lactobacillus delbruekii, Lactobacillus bulgaricus* and *Leuconostoc mesenteroids*. *Lactobacillus* spp. have not been implicated in food poisoning. L*actobacillus bulgaricus* is acid loving organisms and can sour food products. The ability of *Leuconostoc mesenteroides* to cause off-flavour in foods have been reported (Hays and Reister, 1952). Gram negative rods isolated from pearl millet *kunun zaki* were *Proteus vulgaris, Klebsiella aerogenes* and *Escherichia coli*. *Escherichia coli* is the most important member of the coliform group and it's presence in large numbers in foods is generally taken to indicate faecal contamination (Jay, 1987).. *Klebsiella Spp.* have been implicated in histamine associated poisoning and *Proteus vulgaris* causes custard rot (Jay, 1987).

Gram positive cocci isolated were *Staphylococcus aureus, Streptococcus lactis* and *Micrococcus varian*. The presence of *Staphylococcus aureus* in large numbers in foods is undesirable (Jay, 1987). The minimum number of cells of *Staphylococcus aureus* required to produce the minimum level of enterotoxin considered necessary to cause the gastroenteritis in man (1ng/g) differs for substrates and for particular enterotoxin, but the range is from 5 x10^6 to 10^{10} cfu/g or ml (Notermans and Van Otterdijk, 1985). The presence of *Streptococcus lactis* in foods in large numbers may indicate faecal contamination (Jay, 1987). It has been implicated in food spoilage such as souring. Generally, *Micrococcus spp.* are widely distributed in nature and can settle on exposed food and cause spoilage (Jay, 1987).

Moulds isolated from pearl millet *kunun zaki* were *Aspergillus niger, A.fumigatus, A.nidulans* and *Rhizopus arrhizus*. Some *Aspergillus spp.* are pathogenic. *Aspergillus fumigatus* was among fungi isolated in cereal grains and farmers suffer from the inhalation of the spores that cause lung damage and commonly referred to as farmer's lung disease (Hough, 1991). *Rhizopus spp.* can cause food spoilage and occasional causes of serious (and often fatal) infections in

humans (Larone, 1995). The yeasts encountered in *kunun zaki* were *Candida spp.* and *Saccharomyces cerevisiae. Candida* spp. have been implicated in food spoilage especially refrigerated food (Jay, 1987). *Saccharomyces cerevisiae* has been implicated in food spoilage due to its fermentative ability, osmophillic nature, tolerance of acid, tolerance of alcohol and ability to grow at low temperature (Jay, 1987).

CONCLUSION

Addition of malt at 5% level of the grains used for the pearl millet *kunun zaki* preparation did not affect the aroma, colour and overall acceptability of *kunun zaki*. However, the taste and texture of pearl millet *kunun zaki* was improved by the addition of malt from the pearl millet cultivars. Addition of malt did not affect the microbial count of the beverage. *Shigella* and *Salmonella* were not encountered in this study. The population of the microorganisms isolated was not high enough to produce effective dose. However, the need for processors of kunun zaki to adhere to strict hygiene practices (by using sterilized equipment, clean water and environment) is paramount.

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