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Research Article

THE MICROBIOLOGICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF BUBUGN, A TRADITIONAL FERMENTED ETHIOPIAN LOW ALCOHOLIC BEVERAGE

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ABSTRACT

Bubugn is a cereal based traditional fermented Ethiopian low alcoholic beverage. It is one of the traditional fermented beverages used for a drink of holidays, wedding ceremony and also used as a source of income. The aims of this study were to determine the microbiology, microbial contaminants and physicochemical characteristics of Bubugn. Nine Samples of Bubugn were collected from Gondar town in three district areas; Azezo (A), Arada (B) and Kebele 18 (C). Microbial counts and physicochemical analysis were enumerated using standard microbiological methods. The mean value of the pH of the samples was 4.20 ± 0.14 ; and the mean value of moisture content was 45.79 ± 1.35 . The mean crude fat, ash and ethanol contents of Bubugn were 6.67 ± 0.16 , 4.47 ± 0.40 and 1.79 ± 0.13 , respectively. The mean number of total mesophilic aerobic bacteria, yeasts and molds and lactic acid bacteria of Bubugn were 3.74×10^5 cfu/ml, 5.68×10^6 cfu/ml and 7.52×10^5 cfu/ml respectively. Bubugn samples were contaminated by *Shigella* species, *E. coli* and *S. aureus* which could be due to poor hygienic conditions related to washing of preparation material, use of contaminated water and poor personal and domestic hygiene. The collected Bubugn samples were contaminated by different microorganisms and therefore, there should be development of advanced technique to improve the quality.

Key words: Bubugn, Traditional beverage, Low alcoholic beverage.

INTRODUCTION

Traditional fermented beverages are those which are indigenous to a particular area and have been developed by the people

of that area themselves using age-old techniques from locally available agricultural products (Ketema *et al.*, 1998). Fermented products are typically unique and

vary according to regions due to the variation in climate, social patterns, consumption practices and the availability of raw materials (Law *et al.*, 2011). Preparation of these products are still in a small scale and remains as a house art even though the method is simple, highly acceptable and adaptable by the society (Nout, 1993; Blandinob *et al.*, 2003). The Processing techniques of these products are still used in developing countries especially in communities with low income levels (Kebede *et al.*, 2004). Many African foods that are prepared by the action of diverse species of fungi, bacteria and yeasts on plant materials are little known outside their native countries (Kebede, 2007).

Some information is available on the microbiology and biochemical properties of variety of African traditional fermented beverages, including Ethiopian tella and borde (Sahle and Gashe, 1991), Kenya buss (Nout, 1980), Egyptian bouza (Marcos, 1997) and Tanzania togowa, mbege (Kingamkone *et al.*, 1998; Mugula, 2001). Specially, the microbiology and physicochemical properties of many of Ethiopian traditional fermented beverages are not properly written and documented (Getnet and Berhanu, 2016). In Ethiopia, a number of cereal based traditional fermented beverages are prepared. Some of the known beverages are tella, borde, shamita, cheka, keribo, bukire, merissa, koreffe and so forth. Except tella, all of the above listed fermented products are low or non-alcoholic (Kebede *et al.*, 2002). Traditional Ethiopian beverages are very cheap because the cereals and additives used for the production are

obtained from the local agriculture products (Getnet and Berhanu, 2016).

Bubugn, a traditional fermented Ethiopian low alcoholic beverage, is a common drink of North Gondar communities. It is a traditionally fermented beverage usually made from millet, sorghum and barely. This fermented beverage is widely consumed in the area regardless of sex and age. It is an important product because it is consumed as a low-cost meal replacement and therefore provides a cheap food alternative for the low-income group of consumers. It is consumed while actively fermenting and has a short fermentation period, commonly 24 hrs. People in the area prefer Bubugn to high alcoholic traditional and industrial drinks when they have feeling of ill. It is consumed at any season more over extensively consumed during dry season in the local market and work place of farmers. It is widely used for thirst quenching properties. This beverage is produced by the spontaneously fermentation using rudimentary equipment. It is an opaque effervescent whitish-gray to brown in color with thick consistency and sweet taste.

The low ethanol level coupled with crude methods of production under improper sanitary condition may predispose Bubugn to microbial contamination. In the contrary, the acidic nature of the product may make it difficult for some food spoiling and pathogenic microorganisms to survive within it. Still, there is no published scientific work on properties of this traditional fermented product. Therefore, there is a need to investigate the microbiology and physicochemical

properties of Bubugn. In this regard, the objective of this study was to investigate the microbiology, microbial contaminants and physicochemical properties of Bubugn, a traditional fermented Ethiopian beverage.

MATERIAL AND METHOD

Study area and design

The study was conducted in University of Gondar, Northwest part of Ethiopia. Laboratory based experiment was conducted from January 2017 to June 2017 to study the microbiological quality and microbial contaminants of Bubugn.

Collection of samples and sampling technique

Nine samples (400 ml each) of Bubugn were collected in sterile bottles randomly from Bubugn vendors at three localities in Northwest part of Ethiopia, Gondar town, namely (Arada, Azezo and Kebele 18). After transportation of the samples, microbiological analysis, pH, titratable acidity, ash content and moisture content were immediately determined in the laboratory according to Getnet and Berhanu, (2016).

Physicochemical determination

The pH was measured using a digital pH meter after calibration using buffers of pH 4 and 7. With regard to titratable acidity, 5 ml of the Bubugn sample was mixed with 20 ml of distilled water. Three to five drops of phenolphthalein indicator was added in to the mixture. The solution was titrated against 0.5 N NaOH. The amount of lactic acid produced in 100 ml of medium was calculated as percent lactic acid (Fite *et al.*, 1991). Alcohol content was determined using distillation through direct heating and calculated using distillate specific gravity

(Rosendal and Schmidt, 1987). The moisture content was determined by drying 3 g of Bubugn sample in a forced draft oven at $102 \pm 2^\circ\text{C}$ for 3 hrs. The moisture and ash content of the Bubugn was calculated according to Bradley *et al.*, 1993.

For the determination of fat content, 2.0 g sample was solubilized in alcohol (2 ml) and hydrolyzed with 10 ml concentrated hydrochloric acid at $70\text{-}80^\circ\text{C}$ for 40 min in water bath. The hydrolyzed fat was extracted with petroleum ether for 24 hrs. The ether was evaporated from the extract and the fat was dried to constant weight at 100°C for 90 min. The ash content was determined by igniting the pre-dried Bubugn (2.0 g) in a Muffle Furnace at 550°C . The sample was ignited until constant mass achieved (4 hrs) (Ahmed and Kanwal, 2004).

Microbiological analysis of Bubugn

Microbiological analysis was carried on Plate Count Agar, MacConkey Agar, Mannitol Salt Agar, MRS, M17 and Potato Dextrose Agar. All of the media used were oxid. Two and half gm of sample was transferred aseptically to 22.5 ml normal saline and homogenized for 1 min by a homogenizer. The homogenate was then serially diluted (10^{-1} to 10^{-10}) and aliquots of 0.1 ml from appropriate dilutions was spread-plated in triplicate on pre-dried plates of Plate Count Agar, MacConkey Agar, Mannitol Salt Agar, MRS, M17 and Potato Dextrose Agar. The culture of Plate count agar, MacConkey agar and Mannitol Salt Agar plates were incubated at 30°C to 35°C for 24-48hrs.

Colonies of lactic acid bacteria were counted on MRS and M-17 Agar plates after

anaerobic incubation in Gas Pak jars at 30°C for 48 to 72 hrs. Yeast and mold colonies were counted on PDA at 25°C for 3 to 5 days (Kebede *et al.*, 2002). Colony counts were made using colony counter (R000102371, UK). The mean of triplicate results were then recorded as the colony count (Lateef, 2004).

Characterization and Identification of bacterial Isolates

Distinct colonies from the spread plates were streaked onto fresh sterile nutrient agar plates and incubated at the appropriate temperature and time to obtain the pure colonies. The morphological (microscopic) characteristics of each pure bacterium colony was studied with on the pigmentation, color, shape edge and elevation, optical characteristics that is opaque, translucent or transparent the colony surface and constancy. Gram staining reaction, spore stain reaction as well as motility test was also done. Biochemical tests employed in the identification of the bacterial isolates are catalase, indole test, methyl red test, citrate utilization test, mannitol test, urease test, H₂S, coagulase and gas in glucose. These tests performed according to the methods described by Cheesbrough (2005), Ochei and Kolhatkar (2007).

Risk assessment of Bubugn

Hundred ml of bubugn sample was mixed with 200 ml of distilled water and filtered with filter paper. Five ml of the mixture was taken to the test tubes and autoclaved. On the autoclaved sample, refreshed loop full of standard pathogenic bacteria: *S. flexneri* (ATCC1202) *S. typhi* (clinical isolate), *E. coli* (ATCC25922), *S. pneumoniae* (ATCC14619) and *S. aureus* (ATCC 25923) were deliberately inoculated and then incubated at 37°C for 48 hrs.

Autoclaved sample without inoculation of pathogenic bacteria was used as negative control. After 48 hrs incubation period, a loop full from the sample was taken and streaked on Muller Hinton agar and incubated at 37°C for 24 hrs to check whether the beverage was used as a medium for the pathogenic microorganisms or not according to Getnet and Berhanu (2016).

RESULTS

Results of pH determination showed that all the samples were acidic in nature. The mean value of the pH of bubugn samples were 4.20±0.14. The moisture content of Bubugn was also measured and the mean value was 45.79 ±1.35. There was no great variation on the pH range and moisture content of the collected samples from the vendors. The pH and moisture content of Bubugn collected from three Bubugn vendors is shown in Table 1.

Table 1. Mean value of the physicochemical characteristics of Bubugn collected from three Bubugn vendors.

Physicochemical analysis	Samples			
	A	B	C	Average
pH	4.21±0.10 ^a	4.17±0.17 ^a	4.20±0.20 ^a	4.20±0.14

Moisture (%)	45.30±0.90 ^a	45.82±1.4 ^a	46.27±1.96 ^a	45.79±1.35
TA (%lactic acid)	0.69±0.02 ^a	0.72±0.10 ^b	0.79±0.10 ^b	0.73±0.40
Fat (%)	6.70±0.10 ^{ab}	6.50±0.10 ^a	6.80±0.10 ^b	6.67±0.16
Ash (%)	4.00±0.10 ^a	4.50±0.10 ^b	4.90±0.10 ^c	4.47±0.40
Ethanol (%)	1.80±0.10 ^{ab}	1.68±0.10 ^a	1.90±0.10 ^b	1.79±0.13

Key: A= Azezo; B= Arada; C=Kebele 18

Values are means of triplicate determinations; Values within the same row followed by different superscripts are significantly different at (P<0.05). Mean of total mesophilic aerobic bacteria grow on Bubugn sample was 3.74×10^5 cfu/ml; the

means counts of yeasts and molds was 5.68×10^6 cfu/ml. Lactic acid bacteria was also isolated from Bubugn in a relatively large amount of 7.52×10^5 cfu/ml. The microbial counts (cfu/ml) of Bubugn collected from three Bubugn vendors are shown in Table 2.

Table 2. Microbial counts (cfu/ml) in Bubugn, collected from three Bubugn vendors.

Sample	PCA	MCK	MSA	MRS	M17	PDA
A	3.51×10^5	7.80×10^1	4.70×10^2	5.2×10^6	8.4×10^5	6.0×10^6
B	4.40×10^5	1.52×10^1	4.30×10^2	4.32×10^6	6.98×10^5	4.20×10^6
C	3.30×10^5	1.80×10^1	3.10×10^2	4.76×10^6	7.20×10^5	7.4×10^6
Mean	3.74×10^5	3.70×10^1	4.0×10^2	4.76×10^6	7.52×10^5	5.68×10^6

Key: PCA= Plate Count Agar; PDA= Potato Dextrose Agar; MCA=MacConkey Agar; MSA=Mannitol Salt Agar; MRS= de Man, Rogosa Sharp.

Staphylococcus aureus and *E. coli* were isolated from samples A, while samples B contained *S. aureus* and *Shigella*. *Staphylococcus aureus* and *E. coli* were isolated from sample C. The bacteria species *S. aureus* had the highest occurrence followed by *E. coli* and while *Shigella* species had the list occurrence. Microbial

contaminants isolated from Bubugn samples are showed in Table 3.

Table 3. Microbial contaminants isolated from Bubugn collected from Bubugn vendors

Microorganism	Samples		
	A	B	C
<i>S. aureus</i>	+	+	+
<i>E. coli</i>	+	-	+

<i>Shigella spp.</i>	-	+	-
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Key: + presence; - Absence

Risk assessment of Bubugn

The standard pathogenic bacteria used for the risk assessment test, *S. pneumoniae* (ATCC14619), (*ATCC 25923*), *E. coli* (ATCC25922), *S. flexneri* (ATCC 12022) and *S. typhi* (clinical isolate) were

well grown on Bubugn samples. *Staphylococcus aureus* was unable to grow. The risk assessment of pathogenic bacteria in Bubugn is showed in Table 4.

Table 4. Risk assessment of pathogenic bacteria in Bubugn sample

Standard pathogenic bacteria	Bubugn
<i>S. flexneri</i> (ATCC 12022)	+
<i>E. coli</i> (ATCC25922)	+
<i>S. pneumoniae</i> (ATCC146190)	+
<i>S. aureus</i> (ATCC 25923)	-
<i>S. typhi</i> (clinical isolate)	+

Key: + grow well; - unable to grow

DISCUSSION

Bubugn is a fermented beverage that is widely consumed for its thirst quenching property. Due to the traditional brewing, unhygienic processing material and the illiteracy level of processing personnel's involved in Bubugn production makes it expose for contamination. The moisture content of Bubugn is highly lower than the many of traditional fermented Ethiopian beverage; koreffe (96-98%) (Getnet and Berhanu, 2016); tella (96%) (Sahle and Gashe, 1991). Even though the moisture content is almost half less than the above listed two fermented products, the risk assessment and microbial contaminant experiment indicated that Bubugn is highly exposable and suitable for the growth of microorganisms. This might be due to the short fermentation period was unable to extract chemical substances which inhibit

potentially pathogenic microorganisms and also due low level of lactic acid production.

The mean pH value of Bubugn is almost similar to other low and nonalcoholic fermented Ethiopian beverage like Ethiopian borde (4.1), Ethiopian tella (4.3 to 4.51), (Getachew, 2015). The average pH value of borde from the open markets of five localities in Southern Ethiopia was 3.92 ± 0.14 (Kebede *et al.*, 2002); Mean value of the pH of korefe samples collected from both vendors and prepared korefe is 4.15 ± 0.34 (Getnet and Berhanu, 2016). These different studies showed that pH of Ethiopian fermented low alcoholic beverage range are between 4 and 5. This low pH value of Bubugn and other fermented beverages could be due to the involvement of large number of lactic acid bacteria through the fermentation process. The acidic nature of the sample may also be due to the fact that the Bubugn might have certain

metabolites that could bring about reduction in pH. Similarly local drinks with acidic pH value have reported for zobo and for orange juice product (Lateef, 2004).

Although these classes of beverage are acidic in nature, the acidity tends to decrease with increase in fermentation period resulting in to spoilage. Generally, the low pH in Bubugn was in agreement with other cereal based fermented beverages like Ethiopian tella (Sahle and Gashe, 1991), Kenyan kirario (Kunyanga *et al.*, 2009), Ugandan kwete (Namugumya and Muyanja, 2009), Zimbabwean mukumbi (Kock *et al.*, 2008), Ethiopian borde (Kebede *et al.*, 2002) and shamita (Mogessie and Tetemke, 1995). Bubugn and other low alcoholic beverages such as Ethiopian koreffe, borde and shamita have been reported to a high nutritional value because of the raw material which they made and in some traditional beverages spices are usually added in small quantity to improve taste, flavor and aroma (Getnet and Berhanu, 2016). And this is supportive for the finding of the current research. The raw materials may contain a high level of microbial impurities and could be source of spoilage and potentially pathogenic microorganisms (Adeyami and Umar, 1994; Bibek, 2001).

The pH value of Bubugn usually too low to allow the growth of pathogenic microorganisms, but the presence of *E.coli*, *Shigella* species and *S. aureus* could be matter of serious concern. *E.coli* is an important member of the coliform group of bacteria and is part of the normal flora of the intestine of humans and vertebrates. It has been shown that the same strain of *E.coli* gastroenteritis and urinary disease tract

infection as well diarrhea in infant and young children (Kolawole *et al.*, 2011).

There was high population of microorganisms in Bubugn, total mesophilic aerobic bacteria is 3.74×10^5 cfu/ml. The presence of high microbial loads was an indication of poor hygienic of the processes, producers, raw materials and also utensils. The presence of these fecal contaminations may be attributed to improper sanitation condition during processing and unhygienic water supply. High counts of total mesophilic aerobic bacteria may trigger health problem which provides that there are potential pathogenic strains among the strains including *E.coli* and *Salmonella* (Rashid, 2013). *Shigella* species cause diarrhea which often is bloody. The presence of this pathogen even in small number could render beverage unsuitable for human consumption. Contamination by this pathogen could have occurred during processing. Packaging and hawking do not take necessary precautions, and as such contamination could be very prominent (Rashid, 2013).

The mean alcoholic content of Bubugn is 1.79 ± 0.13 (v/v). The mean alcoholic content of tella, koreffe, borde and teji varies from report to report (Getachew, 2015). In the year 1991, Sahle and Gashe, reported that a good quality of tella consists the final ethanol content is in the range of 2-8 % (v/v). The mean value of ethanol content of koreffe was 2.706 ± 0.7 (Getnet and Birhanu, 2016). The difference in alcoholic content of traditional alcoholic beverages is due to the difference in way of preparation as well as fermentation. Moreover, conditions such as temperature,

aeration, and strains of the microorganisms obviously affect the level of alcohols. As the beverage stored a certain time, the alcoholic content is increased. This may be due to the increase in the population of the organisms. *Saccharomyces cerevisiae* and *Lactobacillus* species enhance the fermentation process and decreases the amounts of reducing sugar and total carbohydrate (Berihu *et al.*, 2015).

In case of risk assessment, the entire Gram negative bacteria were grown well. From the Gram positive groups *S. aureus* (ATCC 25923) was unable to grow but *S. pneumonia* (ATCC146190) was able to grow like the tested Gram negative bacteria. Getnet and Berhanu (2016) reported that after autoclaving of koreffe sample and deliberate inoculation of standard and clinically isolated bacteria only Gram negative groups were grown well but the entire tested Gram positive groups did not grow. Traditional alcoholic beverages have low pH and lipophilic extracts that inhibits proliferation of some pathogenic bacteria (Blandinob *et al.*, 2003; Mogessie, 2006; Chelule *et al.*, 2010; Anteneh *et al.*, 2011).

The low pH and lipophilic extracts highly affect Gram positive bacteria but Gram negative bacteria are not susceptible which consist of lipopolysaccharide and outer membrane that makes them impermeable to lipophilic extracts (Tortora and Funke, 2001). Even though *S. pneumoniae* (ATCC146190) is Gram positive bacteria, it was able to grow on the autoclaved Bubugn. This may be due to the fast fermentation time of Bubugn unable to extract many of the lipophilic chemical substances. And also such strain of *S. pneumoniae* may be able to withstand such

level of acid. In the contrary *S. aureus* (ATCC 25923) was unable to grow on the autoclaved Bubugn sample. This strain of *S. aureus* could be sensitive for such a low pH. Gram positive bacteria are more susceptible because of having only an outer peptidoglycan layer which is not having effective permeability barrier (Tortora and Funke, 2001).

CONCLUSION

Microbial profile and microbial contaminants of traditional beverage studies must be extended very less known indigenous fermented beverages, the popularity of which is restricted only to the areas of origin. This may enable to come across novel microorganisms with novel metabolites, which subsequently may have industrial application.

The investigated samples of Bubugn, from the three villages of Gondar town were characterized by presence of indicator microorganisms, *Shigella*, *E. coli* and *S. aureus*. The isolation of indicator organism from the investigated samples is an indication of the poor quality procedure of Bubugn production, filtration and the water used for the process. From the result of this research, it is possible to say that the physicochemical property of Bubugn is similar to other Ethiopian beverages like koreffe, borde, shamita and tella. Generally, further study is necessary on the fermentation process and basic microorganisms involved through the fermentation of Bubugn.

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