SHORT COMMUNICATION

MICROORGANISMS ASSOCIATED WITH STARTER CULTURES OF TRADITIONAL BURUKUTU LIQUOR IN MADAKIYA, KADUNA STATE, NIGERIA.

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INTRODUCTION

Burukutu is an indigenous beverage produced and consumed locally in Nigeria and some African countries. It is produced mainly from starchy grains such as guinea corn, millet and maize. These crops are produced in the tropical regions of Africa and particularly in the Northern Guinea Savanna areas of Nigeria (Ettasoe, 1972; Asiedu, 1987).

Burukutu production involves the processes of malting, mashing, boiling, fermentation and maturation. (Ekundayo, 1969).The microorganisms associated with fermentation include *Saccharomyces cerevisiae. Saccharomyces chavelieria* and *Leuconostoc mesenteroides* (Kolawole, 2007). These microorganisms are cultured in a small pot that is continuously being transferred into a fresh produce. This serves as the starter culture daily and without the starter culture the burukutu will not mature.

Sanni *et al.,* (1999) observed that deteriorating burukutu had a vinegary flavour and off-odour with pronounced characteristics of the deteriorating beverages. They observed decrease in ethanol from 3 % in fresh product to 1 % at the end of 72 hrs of fermentation. They also observed slight decreases in lactic, malic, succinic and formic acids content of the drink. Fermentation has the following advantages: longer keeping quality, variety in flavour and making inedible foods edible, in addition to enhancing nutritional values and decreasing the toxicity of the fermented foods (Odunfa, 1988).

During the fermentation of maize grains, Oyeyiola (1990) oserved that the temperature and moisture increased and the pH decreased. Most amino acids decreased in concentration during the fermentation with glutamic acid being the most abundant. The bacteria played the major role in the fermentation. Sanni & Oso (2006) observed that titratable acidity and alcohol content of "agadagidi" gradually increased till the end of the fermentation while the total soluble solid and pH decreased.

This research was carried out to investigate the microorganisms that are harboured in the starter cultures since the media are rich in moisture and nutrients.

Collection of starter cultures: 500 ml starter cultures of burukutu (produced from *Sorghum bicolor*) were collected in sterile flasks from five different producers (sites 1-5) of burukutu in Madakiya village, Kaduna State, Nigeria. These were transported and analysed in the laboratory.

Alcoholic content: 100 ml of the starter culture was distilled using microdistillation apparatus set at 70 ^{0}C. Alcohol meter was used to determine the percentage alcohol content of the distillate obtained.

Dry matter content: Five grams each of the samples was obtained by scrapping the outside and inside surfaces of the starter culture pots into pre-weighed crucibles and dried in a drier at 100 ^{0}C for 12 hrs. The dried samples were weighed after cooling in a desiccator (AOAC, 1980).

Ash content: 5 grams of each sample was weighed in a small crucible of known weight. The samples were charred in a muffle furnace at 500^{0}C for 3 hours. The ash materials were removed and also cooled. These were kept in a desiccator and reweighed (AOAC, 1980).

Crude protein: 5 g of each dried sample was digested with concentrated H_2SO_4, concentrated NaOH (40 %), K_2SO_4 and $CuSO_4$. 5 ml of the digest was placed in a micro-kjeldahl distillation apparatus and excess concentrated NaOH was added to make the solution strongly alkaline. Ammonia was added into 5 ml of boric acid indicator in a titrating flask. 45 ml of the distillate was collected. Titration was done with 0.01 M HCl. The end point was light green (AOAC, 1980). The pH of the starter cultures was determined immediately after collection with a pH meter (Jenway, Model No. 3505).

Minerals analysis of the samples: The mineral analysis of each sample was carried out to determine magnesium calcium, iron and potassium in the starter cultures using methods of AOAC (1980).

Microbiological determination of the starter cultures: A serial dilution of the starter culture samples (10^4) was inoculated in: nutrient agar, potato dextrose agar and Muller Hinton agar plates to identify the bacterial and fungal isolates. Bacterial identification was carried out using various biochemical tests of Cowan & Steel (2004) and Buchanan & Gibbons (1974). Fungal identification was done using microbiological atlas (Alexopolus & Mims, 1979; Robert & Ellen, 1988) as well as the methods of Sawadogo, *et al.,* (2007). The isolates were identified and characterized by a polyphasic approach based on phenotypic and genotypic methods using carbohydrate fermentation.

There were variations in the alcoholic contents of the starter cultures from 0.7 % in site 4 to 2 % in site 3 which had the highest alcohol.

It therefore indicated that the level of fermentation was higher in that site (4) than the other four as reflected in the pH which was lowest in the third site (3.5) than the other sites. The mineral contents in the five starter culture pots did not differ significantly (Table 1).

The proximate analysis showed that the ash content varied between 4.7 % and 5.6 %, Dry matter was between 6.0 % and 7.3 % while protein had a range of 4.1 % and 4.8 % (Table 2).

Table 3 shows the different microorganisms isolated from the five sites of the starter cultures. Site 5 had the lowest number of microorganisms (11) while site 1 and 4 had the highest (14) microorganisms isolated. The starter cultures had mixed cultures of microorganisms.

Eight microorganisms were isolated in all the sites and one (*Aspergillus flavus*) had a rare appearance in one site.

TABLE 1. ALCOHOLIC CONTENT, pH AND MINERAL ELEMENTS OF THE STARTER CULTURES

	% alcoholic content	Mineral elements (ppm)				
		pH	Mg	Ca	K	Fe
1	1.7	3.8	115	1.32	1.71	11.70
2	1.8	3.9	113	1.47	1.67	10.70
3	2.1	3.5	110	1.38	1.89	10.40
4	0.7	5.3	116	1.30	1.91	8.10
5	1.5	4.5	112	1.19	1.30	9.10

TABLE 2. PROXIMATE ANALYSIS OF THE STARTER CULTURES FROM DIFFERENT SITES

Site	Ash content %	Dry matter %	Crude protein %
1	4.8	6.1	4.1
2	5.6	7.3	4.5
3	4.7	6.0	4.8
4	4.8	6.5	4.3
5	5.0	6.8	4.7

TABLE 3. MICROORGANISMS ISOLATED FROM THE STATER CULTURES

Microorganisms	Sites				
	1	2	3	4	5
Lactobacillus fermentum	+	+	+	+	+
Pediococcus acidilactici	+	+	-	+	-
Lactobacillus delbrueckii	+	+	+	-	+
Acetobacter aceti	-	-	+	+	+
Lactobacillus plantarum	+	-	+	+	+
Lactobacillus brevis	+	+	+	+	-
Streptococcus lactis	+	+	-	+	-
Escherichia coli	+	+	+	+	+
Staphylococcus aureus	+	+	+	+	+
Bacillus subtilis	+	+	+	+	+
Rhizopus stolonifer	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Aspergillus flavus	+	-	-	-	-
Mucor pusilus	+	+	+	+	+
Saccharomyces cerevisiae	+	+	+	+	+
Total	**15**	**13**	**12**	**14**	**11**

The presence of all the 15 microorganisms other than yeast could lead to faster deterioration of the burukutu as they are permanently and consistently present in the starter cultures. Earlier study by Faparusi *et al.,* (2007) found the presence of contaminants in the various stages of burukutu liquor production and indicated that most of the bacteria survived at low acidity. This observation had been confirmed by other researchers (Owuama 1991, Sanni *et al.,* 1999, Orji *et al.,* 2003, Faparusi, 2007, Kolawale *et al.,* 2007, Sawadogo *et al.,* 2007, Achi, 2009). The presence of *E. coli* in all the sites indicate a very low level of hygienic conditions of the environment and the individuals involved in the production and sale of the commodity. The bacteria *S. cerevisiae* appeared in all the sites and could be the predominant fermenter while most of the other microorganisms were there as opportunistic and contaminants from the environment. The bacteria *A. flavus* encountered in site 1 is known to be deleterious to human health. Its presence in the starter culture pot could be dangerous to the consumers of the product. It would be important for the producers to have a time span for the continuous usage of the starter cultures so as not to harbour pathogenic microorganisms in their alcoholic beverages.

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