

The fermentation characteristics of single and mixed yeast cultures during *pito* wort fermentation

¹Avicor, M. N., ¹Saalia, F. K., ²Djameh, C., ³Sinayobye E., ^{3*}Mensah-Brown, H. and ⁴Essilfie, G.

¹Nutrition and Food Science Dept. University of Ghana, ²Food Research Institute, CSIR, Accra, ³Food Process Engineering Dept. University of Ghana, ⁴Crop Science Dept. University of Ghana

Article history

Received: 6 October 2013
Received in revised form:
23 June 2014
Accepted: 25 July 2014

Keywords

Pito
Single and mixed cultures
Fermentation
Volatile aroma compounds

Abstract

Pito is an alcoholic beverage obtained through yeast fermentation of the wort extracted from sorghum malt. The production methods are largely artisanal and the product quality varies from batch to batch even for the same brewer. The use of starter culture is a plausible approach to minimizing the variability in product quality. This work compared the fermentation profile of single yeast and mixed yeast cultures in sorghum wort fermentation during *pito* production. A 2 x 3 factorial design was used to study the quality characteristics of wort pitched with single and mixed Culture yeast strains during 24, 48 and 72 hours alcoholic fermentation. The physicochemical properties, microbial load and sensory characteristics of samples were determined. Fermentation time had significant effects ($p < 0.05$) on the *pito* characteristics. *Pito* color (of 12.5 and 16.5 EBC units) was different for single and mixed cultures respectively. Panelists perceived the colours of *pito* obtained from single yeast culture fermentation to be different from that obtained from mixed culture fermentation. Volatile aroma compounds identified during the fermentation period included esters (4), aldehydes (3), organic acids (2), alcohols (3) and a diacetyl. Both single and mixed cultures showed similar characteristics during wort fermentation.

© All Rights Reserved

Introduction

Pito is a traditional alcoholic beverage brewed and drunk by people in the West African sub-region (Demuyakor and Ohta, 1992; Sanni, 1993). It is golden yellow to dark brown in colour with taste varying from slightly sweet to very sour. It contains lactic acid, sugars, amino acids, 2-3% alcohol and some vitamins and proteins (Bansa, 1990). Brewing of *pito* involves steeping of sorghum grains, germination, drying, milling, wort extraction and fermentation (Demuyakor and Ohta, 1992).

Fermentation of *Dagarti pito* is done by inoculation of the wort using dried yeast cells (*dambila*) from a previous brew or by using a portion of the previous brew as inoculum (Sefa-Dedeh and Asante, 1988; Demuyakor and Ohta, 1992). The use of *Dambila* (or backslopping) as starter for fermentations in the *pito* brewing process does not necessarily ensure consistent product quality since it is a mixture of different strains (Sefa-Dedeh *et al.*, 1999). It has been established that *Dambila* is a top fermenting strain while those trapped within the woven belt are bottom fermenting (Bansah, 1990; Demuyakor and Ohta, 1992). The use of starter cultures could improve

the quality and predictability of the alcoholic fermentation process. The fermentation time, production of flavors, visual appearance, texture and other attributes that could help assure product quality and consistency would also be more predictable with the use of known starter cultures.

Yeast (*Saccharomyces cerevisiae*) and Lactic acid bacteria (LAB) have been identified as the predominant microorganisms in African opaque beers (Demuyakor and Ohta, 1992; Sefa-Dedeh *et al.*, 1999; Glover *et al.*, 2005). They are involved in the spontaneous acidification of *dolo* and *pito* wort, with a genetic diversity at strains level (Sawadogo-Lingani *et al.*, 2007). Studies on identification of the fermenting microorganisms and characterization of the *pito* production processes have been reported (Bansah, 1990) as well as its microbiological safety. The fermenting microorganisms are known to be able to produce a wide range of compounds which contribute to the taste, flavour, colour, texture, consistency, quality and safety of the product (Ayad *et al.*, 2004). This study compared the fermentation profile of single and mixed yeast cultures used in wort fermentation during *pito* production.

*Corresponding author.
Email:

Materials and Methods

Raw material preparation

A variety of sorghum popularly referred to as *chire* (or *kyere*) by *Dagaarti pito* brewers was used for malting and brewing of *pito*. The sorghum grains were obtained from a local supplier and kept in a hermetic grain bag (SuperGrainBagII™) until used for malting. The dried malt was milled for three 30 second periods to obtain fine grits and flour (i.e. from receiver up to 500 micron sieve) composition of 80% using a water cooled laboratory mill IKAM20 (IKA Labortechnik, Janke & Kunkel GmbH & Co. Kg, Staufen, Germany). A mashing process as described by Agu and Palmer, (1997) was followed with addition of calcium ions in the form of calcium chloride at 200 ppm to the mash liquor. The mash was stood for 18 hrs to sour at ambient temperature of 29°C, filtered and boiled to obtain the sweet wort.

Experimental design

The wort was boiled, cooled and fermented following a 2 x 3 factorial design. The factors and their respective levels were: Inoculum type (Single and Mixed Yeast cultures) and Fermentation time (24, 48 and 72 hours). A proprietary pure yeast strain (Brewers' Yeast) was used as the single culture and the dried top foamy portion (*Dambila*) from a previous brew (obtained from a traditional brewer) was used as the mixed culture.

Determination of fermentation characteristics of samples

During and after the stipulated fermentation time, samples were taken and analysed for quality indices as outlined below. All determinations were done in triplicate.

Physicochemical analysis

°Brix, °Plato and specific gravity (S.G.)

°Brix of the wort samples was determined using a refractometer (AOAC, 1992).

pH and Total acidity

pH was determined according to AOAC (1992) with a digital pH meter (OAKTON deluxe waterproof pH/ Conductivity meter kit, model No. 35630-62, Japan). The titrimetric procedure of determining Total acidity in wort as detailed in EBC Analytica, (1987) was also followed.

Wort colour (°EBC) and turbidity

Wort colour was determined by Spectrophotometry as described by EBC Analytica, (1987) and the result

expressed in EBC units to 2 significant figures. Wort turbidity was determined using a Hanna turbidity meter, model H193703-11 and expressed as turbidity in FTU (EBC Analytica, 1987).

Free amino nitrogen (FAN), reducing sugars and alcohol

FAN was determined using the International Ninhydrin Colorimetric Method (Analytica EBC, Fourth edition, 1987), while reducing sugars were determined using Lane and Eynon's method. Percent alcohol in the fermenting *pito* samples, was calculated and expressed as Percent Alcohol by Volume (%ABV) using the formula:

$$\text{Percent Alcohol by weight (\%ABW)} = (\text{O.G} - \text{F.G}) \times f_1$$

$$\text{Percent Alcohol by Volume (\%ABV)} = \%ABW \times f_2$$

Where O.G. = Original gravity

F.G. = Final gravity

f_1 and f_2 are factors which are 105 and 1.25 respectively.

Microbial flora during fermentation of *pito*

Enumeration of aerobic mesophiles was carried out on Plate Count Agar (PCA, Merck 5463, Darmstadt, Germany) and incubated at 30°C for 3 days. The LAB micropopulation was enumerated on deMan Rogosa and Sharpe medium (MRS Oxoid) (deMan *et al.*, 1960) after 10-fold dilution of the sample and incubated anaerobically in an anaerobic jar at 30°C for 5 days. Yeasts were enumerated on Oxytetracycline-Glucose Yeast Extract Agar (OGYEA, Oxoid CM0545) containing 4 ml oxytetracycline (Oxytetracycline Selective Supplement Oxoid) and incubated at 25°C for 5 days.

Volatile analyses with GC-FID

Identification and quantification of volatile compounds in the *pito* samples were done according to a protocol described by Duarte *et al.*, (2010) with some modifications. In order to identify the major volatile compounds, the samples were analyzed directly without any pretreatment. A Varian CP-3800 gas chromatograph equipped with a Split/Splitless injector, a flame ionization detector, and a capillary column (30 m x 0.25 mm i.d., 2.5 µm film thickness; Chrompack) coated with CP-Wax 52 CB was used. Nitrogen was used as the carrier gas at a constant flow rate of 2.0 mL min⁻¹. Injections of 1 µL were made in the splitless mode (vent time, 60 s) and the volatile compounds were identified by comparing the retention times of the samples with those of standard compounds.

Table 1. Physicochemical characteristics of the fermenting *pito* wort

Wort parameter	Yeast culture	FERMENTATION TIME (HR)			
		0	24	48	72
Specific Gravity (SG)	Single	1.0416±0.01	1.0244±0.03	1.0204±0.01	1.0204±0.01
	Mixed	1.0416±0.01	1.0331±0.02	1.0219±0.01	1.0206±0.04
Brix (%)	Single	10.4±0.14	6.0±0.30	5.2±0.10	5.1±0.11
	Mixed	10.4±0.14	7.9±0.37	5.5±0.14	5.3±0.11
pH	Single	3.62±0.01	3.54±0.03	3.45±0.01	3.43±0.01
	Mixed	3.62±0.01	3.6±0.01	3.52±0.02	3.50±0.01
Acidity (mg lactic acid/L)	Single	0.64±0.06	0.74±1.0	0.98±0.09	1.18±0.07
	Mixed	0.71±0.06	0.87±0.08	1.02±0.07	1.17±0.06
FAN	Single	126±5	106±6	51±9	38±6
	Mixed	128±5	113±5	55±3	45±7
Color EBC	Single	22±0.77	16±0.91	13±0.71	12.5±0.21
	Mixed	22±0.77	18.5±0.83	17.5±0.63	16.5±0.35
Reducing Sugars	Single	86±5	43±3	20±2	17±3
	Mixed	86±5	66.8	22±3	18±2
Alcohol ABC(%)	Single	ND	2.26±0.06	2.78±0.03	2.78±0.02
	Mixed	ND	1.10±0.02	2.65±0.03	2.73±0.01

Sensory analysis of fermented *pito* wort

Panelists (n=15) were randomly recruited from the University of Ghana campus. Criteria for recruitment were that panelists were regular consumers of *Pito* and that they were very familiar with the characteristics of *Pito*. Consumers were asked to evaluate the samples based on the clarity, color, aroma, taste, palate fullness and alcohol strength using a line scale for attribute intensity while a 5-point hedonic scale (1 = very good, 3 = fair, and 5 = very poor) was used to indicate the degree of acceptability.

Statistical analysis

The results were analyzed using Analysis of Variance (ANOVA) procedures in the StatGraphics plus software for Windows Centurion version 15 (Graphic Software System, S.T.C.C., Inc., Rockville, Maryland, USA). Significance was set at $p < 0.05$ for all analyses. Fisher's least significant difference (LSD) procedure was further used to separate the means of treatments.

Results and Discussion

Pito fermentation characteristics

°Brix

The *°Brix* showed significant decreases with fermentation time (Table 1), but there were no significant differences ($p < 0.05$) between inoculum type. The *°Brix* decreased from an initial 10.4 to 5.2 in 48 hours of fermentation for both single and mixed cultures. The decreasing trends in *°Brix* was expected because as the wort sugar is converted into alcohol, there is a decrease in sugars the appearance of ethanol both of which contribute to the decline in specific gravity (Briggs *et al.*, 2004). Moreover, as the wort

is fermented, the simpler sugars are converted into ethyl alcohol and carbon dioxide, and consequently the solids content of the wort progressively decline until fermentation is complete.

No further decline in *°Brix* was observed from 48 to 72 hours indicating the end of fermentation for both inoculum types (Table 1). A sharper decrease in *°Brix* was recorded for Single culture in the first 24 hours of fermentation indicating a more vigorous utilization rate of wort solids than for the mixed culture fermentations. This observation could be explained that in the mixed culture fermenting wort, competition by other organisms for nutrients impede environment acclimatization, utilization and growth of wort components (Hesseltine, 1992).

pH

Both the fermentation time and inoculum type, significantly ($p < 0.05$) influenced pH of the fermenting wort. An increase in fermentation time up to 48 hours led to a decrease in pH for both inoculum types. There was a sharp drop in pH as fermentation progressed with Single Yeast culture from 3.62 to pH 3.54 in 24 hours and, a further decline to 3.45 during 48 hours of fermentation where the pH remained constant even up to 72 hours. The drop in pH during fermentation by the mixed culture on the other hand was more gradual, falling from 3.62 to 3.6 in 24 hours and further to 3.52 in 48 hours. The drop in pH is explained by the accumulation of organic acids during the lactic acid fermentation. Organic acids including succinate, lactate, and acetate are normal products of the metabolism of brewing yeast and their secretion contributes to the characteristic drop in pH that occurs during fermentation. Similar trends in pH have been observed by other authors (Muyanja

Table 2. Volatile compounds found in fermenting *pito* using single and mixed cultures

No.	Volatile Compound	Fermentation time (hrs.)					
		Single culture			Mixed culture		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1	Ethyl acetate	✓	*	✓	✓	*	*
2	2,3-Butanedione	✓	*	✓	✓	*	*
3	Ethyl butyrate	*	✓	*	*	*	*
4	3-Methylbutanol	*	✓	*	✓	*	✓
5	Ethyl caproate	*	*	*	*	*	✓
6	Ethyl caprylate	✓	✓	✓	✓	✓	✓
7	Benzaldehyde	✓	✓	*	✓	*	✓
8	Isobutyraldehyde	✓	✓	✓	✓	*	*
9	Acetaldehyde	✓	✓	✓	✓	*	✓
10	Butyric acid	✓	✓	✓	✓	✓	✓
11	Isovaleric acid	*	*	*	✓	*	*
12	Geraniol	✓	✓	✓	✓	✓	✓
13	Guaiacol	✓	*	✓	✓	*	✓

✓ = Present, * = Absent

et al., 2003; Sawadogo-Lingani, *et al.*, 2007) in *pito* and similar products.

Titrateable acidity

There were no significant differences between inoculum types on Total acidity. During fermentation time up to 72 hours there was increase in Total acidity from 0.64% to 1.18% and 0.71% to 1.17% for single and mixed cultures respectively (Table 1). These values are in agreement with similar reported studies (Demuyakor and Ohta, 1993; Sawadogo-Lingani *et al.*, 2007) on *dolo* and *pito*.

Wort colour (°EBC)

Both fermentation time and inoculum type had significant effects ($p < 0.05$) on the colour of the product. However, the reduction of colour from 0 to 72 hours of fermentation for single culture was greater than for the mixed culture fermentation (Table 1). There was a decrease in colour from 22.5 to 12.5 EBC units at the end of fermentation for single culture fermentation. A more gradual decrease was shown by mixed culture from 22 to 16.5 EBC units at the end of fermentation. For the single culture fermentation, there was a significant change in colour during the first 24 hours of fermentation.

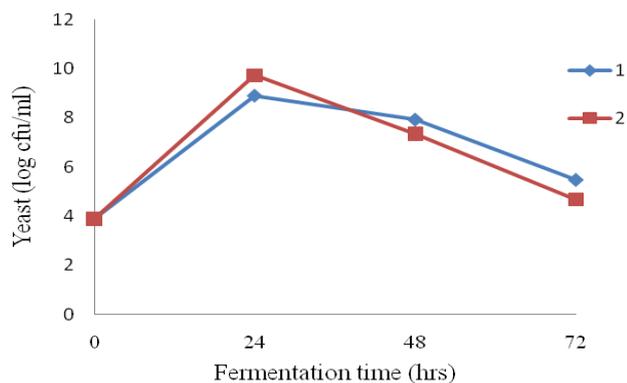
Free amino nitrogen (FAN)

Free amino nitrogen (127.5mg/l) was available

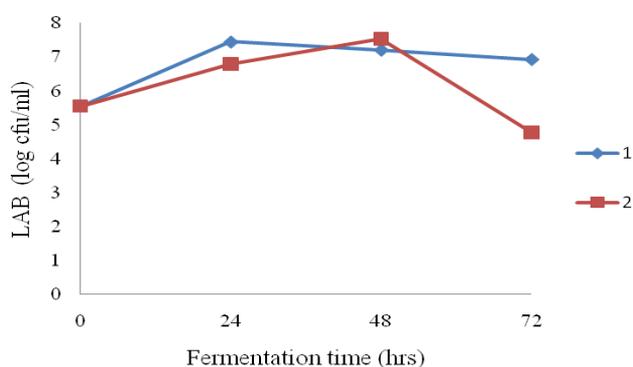
for fermentation by the starter yeast cultures (ie both inoculum types) as shown in Table 1. Inoculum type and fermentation time had significant effects on FAN. Fermentation drastically decreased the FAN levels in the wort for all samples to 37.5 and 44.5 mg/l for single and mixed cultures respectively in 72 hours. Much of the inoculum effect on FAN content was observed within 48 hours of fermentation where levels were reduced by more than 50% (50.5 and 54.5 mg/l for single and mixed cultures respectively). According to Shimizu *et al.* (2002), FAN affects a range of other fermentation factors, such as cell growth, biomass, viability, pH, and attenuation rate. However, high levels of FAN also serve as a recipe for spoilage organisms to feed on. It is therefore necessary that as much of it is used by yeast during fermentation.

Reducing sugars

Reducing sugars content in the wort is very important in fermentation since yeasts convert them into alcohol and CO₂. The trend in depletion of reducing sugars is shown in Table 1. There was a general decrease in reducing sugars content during fermentation. This trend has also been reported by other investigators (Demuyakor and Ohta, 1993; Villicana and Saldivar, 2004) in *pito* and similar products such as sorghum beer and barley wort. Inoculum types were not significantly different in



Inoculum: 1 = Single culture 2 = Mixed Culture
Figure 1. Yeast counts in fermenting *pito* pitched with single and mixed cultures



Inoculum: 1 = Single culture 2 = Mixed Culture
Figure 2. Lactic acid bacteria (LAB) counts in fermenting *pito* pitched with single and mixed cultures

their effect on decreasing the content of reducing sugars. However, fermentation time had a significant ($p < 0.05$) effect on the reducing sugars content. Reducing sugars content decreased from 9.39 mg maltose/100 ml at the start of fermentation to 1.2 and 1.02 mg maltose/100ml in 72 hours for single and mixed cultures respectively. The decrease in reducing sugars in the single culture fermenting wort was more rapid within the first 48 hours than for the mixed culture.

Percent alcohol by volume (% ABV)

An increase in alcohol (%ABV) content was observed for all *pito* samples throughout the fermentation period (Table 1). There was no significant difference between inoculum type on %ABV. %ABV reached a maximum of 2.78 in 72 hours for both yeast culture types. The alcohol contents obtained are in agreement with Briggs *et al.* (2004) who reported values to be within 2.5 to 4.5%. Fermentation time had significant ($p < 0.05$) effects on the alcohol content of *pito*. The highest alcohol content was obtained within 48 hours and remained constant to the end of fermentation (72 hours). The trend of alcohol production was however faster with the single culture than for mixed culture (Table 1).

Microbial counts in *pito*

The mean yeast counts during fermentation are presented in Figure 1. The yeast population significantly increased during fermentation but dropped after 24 hours for worts pitched with either single or mixed cultures. Inoculum type did not have a significant ($p < 0.05$) effect on the yeast population during fermentation. A yeast count of 3.89 log cfu/ml at start of fermentation increased to 8.89 log cfu/ml for single culture and 9.72 log cfu/ml for mixed culture. A decline to 5.48 and 4.67 log cfu/ml was however detected in single and mixed cultures respectively. This is typical of yeast growth cycle indicating the utilization of reducing sugars and other nutrients such as FAN to a point of decline. Similar mean counts have been reported by Demuyakor and Ohta (1993); and Gasseem (2002) in *pito* and sobia respectively. Mean lactic acid bacteria counts (LAB) also followed a similar trend as yeast, increasing to 7.45 log cfu/ml and 6.79 log cfu/ml (24 hours) for single and mixed cultures respectively after which it decreased to 6.93 and 4.77 log cfu/ml was observed in 72 hours (Figure 2). Levels of LAB in *pito* are known to impart the sour taste and acidic nature characteristic of the product.

Aroma volatile compounds in *pito*

The principal flavour metabolites found in alcoholic beverages particularly beer are aliphatic alcohols, aldehydes, organic and fatty acids and esters of alcohols and fatty acids. These are formed as by-products of the metabolism of sugars and amino acids (Briggs *et al.*, 2004). In this work, 4 esters, 3 aldehydes, 2 fatty acids, 3 alcohols and a diacetyl were identified during the fermentation process. The flavor volatile compounds identified in this work are presented in Table 2.

Esters

In *pito* fermentation, ethyl acetate, the most abundant ester found in beer was identified during 24 and 72 hour fermentation in single culture and found only during 24 hours of mixed culture fermentation. Ethyl butyrate, was also identified only in fermentation by single culture in 48 hours. Ethyl caproate, was in mixed cultured fermentation after 72 hours. Ethyl caprylate on the other hand was identified in all the samples throughout the fermentation time. Demuyakor and Ohta (1993) also found similar esters in *pito*. According to Briggs *et al.* (2004) peak ester concentrations are reached after the formation of higher alcohols has ceased.

Acids

Butyric acid was identified in all the *Pito*

Table 3. Scores of sensory attributes of *Pito* fermented with single and mixed culture starters

Sensory attributes						
Sample	Clarity	Colour	Aroma	Taste	Palate fullness	Alcohol strength
SC	7.09±1.00 ^a	2.59±1.26 ^{aa}	5.04±2.23 ^b	5.77±2.21 ^c	6.51±1.79 ^{cd}	5.57±2.17 ^e
MC	7.43±1.08 ^a	7.15±1.66 ^{ab}	6.10±2.33 ^b	6.10±2.75 ^c	6.63±2.08 ^{cd}	6.72±1.51 ^e

*Values within the column followed by different letters are significant at $p < 0.05$

* SC - Single Culture (Brewer's Yeast) MC - Mixed Culture (dambelli)

Table 4. Attribute and Overall acceptability of *pito* pitched with single and mixed culture starters

Sample	Clarity	Colour	Aroma	Taste	Palate fullness	Alcohol strength	Overall Acceptability
SC	2.2	2.4	2.3	2.9	2.2	2.8	2.3
MC	2.7	2.3	1.9	2.0	2.2	2.1	2.1

* SC - Single Culture, Brewer's Yeast MC- Mixed Culture, (Dambelli)

samples and throughout the 72-hour fermentation period. Isovaleric acid was only identified in 24 hour fermentation with mixed culture.

Carbonyl compounds

The carbonyl compounds found in *pito* were acetaldehyde, benzaldehyde and isobutyraldehyde. Acetaldehyde is the immediate precursor of ethanol and formed during the early to mid stages of fermentation and thereafter it declines to a low level. It was identified in all samples except 48-hour mixed culture fermentation. Isobutyraldehyde was not found in the mixed culture sample after 24 hours. Benzaldehyde was not found in 72 and 48 hours of fermentation with single and mixed cultures respectively. Some of these compounds have been reported by Demuyakor and Ohta (1993) to be present in *pito*. The effects of aldehydes on finished beer are a grassy aroma and a papery taste. As a group, these generally make a negative contribution to beer flavor and aroma (Briggs *et al.*, 2004). The principal causes of high acetaldehyde concentrations in beer as indicated by Geiger and Piendl (1976) are the use of poor quality pitching yeast, excessive wort oxygenation, unduly high fermentation temperature and excessive pitching rates.

Higher alcohols

In addition to ethanol, several other alcohols may be found in beer and related products, and may contribute significantly to flavor. The higher alcohols identified in *pito* were 3-methylbutanol, geraniol and guaiacol. 3-methylbutanol was present in only 48-hour single culture fermentations. Guaiacol was

absent in 48 hours of both yeast type fermentation, while geraniol was present in all samples. Alcohols achieve maximum concentrations in fermenting wort at a time roughly coincident with the point at which free amino nitrogen falls to a minimum (Briggs *et al.*, 2004). The composition of the wort, in particular the amino nitrogen content, yeast strain and fermentation temperature affect the production of higher alcohols in the beer.

Diacetyl

The only diacetyl, found in *pito* samples was 2,3 Butanedione. In fermentation with single culture, it was present in 24 and 72 hours, while it was identified in only 24-hour fermentation using mixed culture.

Consumer acceptability of *pito*

The *pito* obtained with single and mixed cultures after 48 hours were evaluated for consumer acceptability using 15 panelists who were regular consumers and very familiar with the characteristics of the product on a 5-point hedonic scale (1 = very good, 3 = fair, and 5 = very poor) preference test. Attribute intensity was also determined with the use of a line scale (1 to 10). The level of preference in terms of clarity (clear to hazy), colour (light brown to dark brown), aroma (weak to strong), taste (sweet to sour), palate fullness (thin to full) and alcohol strength (low to high) from each of the 48-hour fermented *pito* is outlined in Table 3. The attribute and overall acceptance are also shown on Table 4.

With respect to clarity, aroma, taste, palate fullness, alcohol strength, *pito* obtained using the

single culture (SC) and the mixed culture (MC) yeasts fermentation were not significantly different from each other. They were all hazy, had an average to slightly strong aroma, and tasted sweet-sour, bodied with moderate alcohol strength. Colour was the only attribute which was significantly different ($p < 0.05$) for the two samples as depicted on Table 3.

Clarity is the absence of haze or degree of cloudiness of a beverage. It was scored 7.09 and 7.43 for SC and MC pito respectively. In western-styled beers, clarity is of upmost importance since most drinkers expect their beer to be bright and clear and may reject cloudy beer. However, same cannot be said about Native African beers such as pito which need to be cloudy to be recognized as such since it is not clarified after fermentation and consumed together with the microflora. *Pito* has a characteristic brown colour which is derived from the raw material (sorghum) through mashing and wort extraction. There was a significant difference in colour of pito made using SC and MC yeasts as perceived by the panelists. SC and MC pito scored 2.59 and 7.15 respectively by panelists, suggesting that the single culture yeasts affected pito by making it lighter brown as opposed to dark brown for mixed culture.

The aroma of beverages is perceived by the release of volatile substances that are by-products of yeast metabolism which vary with cell growth patterns during fermentation. An average aroma was perceived by panelists indicating a not too strong or not too weak aroma for the two beverages. A similar scoring was done for taste. Most African beers are sweet-sour in nature. Palate fullness also known as 'body' or 'mouthfeel' is defined as the tactile sensations perceived at the lining of the mouth, including the tongue and teeth. Panelists did not perceive a difference between pito fermented by SC and MC with regards to palate fullness. There was no significant difference in terms of alcohol strength between the two products which explains that they were all fully attenuated after the fermentation period. Alcohol strength was scored little above average, indicating a moderate strength of the beverage. Evaluation of the samples on a hedonic scale by panelists had SC *pito* graded 2 (good) and 2.7 (fair) for MC *pito* for color. Panelists indicated that they liked the colour and palate fullness of both samples fairly (Table 4) but their aroma as good. In terms of taste and alcohol strength, there was better preference for MC than for SC which scored fairly.

Conclusion

Both single and mixed yeast starter cultures

showed similar characteristics in fermenting wort. To obtain alcohol content characteristic of *Pito* (1 – 3%), fermenting beyond 48 hours is unnecessary for both inocula. Aroma volatile analysis revealed five (5) major groups of compounds comprising esters, organic acids, carbonyl compounds, higher alcohols and diacetyl. Colour of *pito* obtained using Single cultured yeast was lighter (12.5 EBC units) than that obtained using mixed culture (16.5 EBC units) yeast. Panelist generally had a higher preference for pito obtained from mixed culture yeast.

Acknowledgements

We are grateful to the Office of Research, Innovation and Development (ORID) of the University of Ghana Legon, for the Research Grant. We also thank the Food Research Institute of the CSIR, Accra; for granting access to their pilot brewery facility to undertake the studies.

References

- Agu, R.C and Palmer, G.H. 1997. Effect of mashing procedures on some sorghum varieties germinated at different temperatures. *Process Biochemistry* 32 (2): 147-158.
- AOAC – 1992 “Official Methods of Analysis of the Association of Official Analytical Chemists”, (ed. William Horwitz), 13th Edition, pp. 162 – 170.
- Ayad, E.H.E., Nashat, S., El-Sadek, N., Metwaly, H. and El-Soda, M. 2004. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. *Food Microbiology* 21: 715–725.
- Bansa, D. 1990. Traditional brewing of *pito*: process and product characteristics. Accra, Ghana: University of Ghana, MPhil. Thesis.
- Briggs D.E., Boulton C.A., Brookes P.A. and Stevens R. 2004. *Metabolism of Wort by Yeast, Brewing Science and Practice*, New York: CRC Press.
- De Man, J.C., Rogosa, M. and Sharpe, M.E. 1960. A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology* 23: 130-135.
- Demuyakor B. and Ohta Y. 1993. Characteristics of Single and Mixed Culture Fermentation of *Pito* Beer. *Journal of the Science of Food and Agriculture* 62: 401-408.
- Demuyakor, B. and Ohta, Y. 1992. Malt characteristics of *Sorghum vulgare* varieties from Ghana. *Journal of the Science of Food and Agriculture* 50: 110-119.
- Duarte, W.F., Dias, D.R., Oliveira, J.M., Vilanova, M., Teixeira, J.A. de Almeida e Silva, J.B and Schwan, R.F. 2010. Rasbery (*Rubus idaeus* L.) wine: Yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds. *Food Research International* 43: 2303-2314.
- European Brewery Convention (EBC) *Analytica*, 4th edn. Zurich, Brauerei und Getraenke, Rundschau, 1987.

- Gassem, M.A.A. 2002. A microbiological study of Sobia: a fermented beverage in the Western province of Saudi Arabia. *World Journal of Microbiology and Biotechnology*, 18: 173-177.
- Geiger, E. and Piendl, A. 1976. Technological factors in the formation of acetaldehyde during fermentation, *MBAA Tech. Q.* 13: 51.
- Glover, R.L.K., Abaidoo, R.C., Jakobsen, M. and Jespersen, L. 2005. Biodiversity of *Saccharomyces cerevisiae* isolated from a survey of *pito* production sites in various parts of Ghana. *Systematic and Applied Microbiology* 28: 755–761.
- Hesseltine C.W. 1992. Mixed-Culture Fermentations: In Applications of Biotechnology to Traditional Fermented Foods. Report of an Ad Hoc Panel of the Board on Science and Technology for International Development, National Academy Press, Washington, D.C., p. 52.
- Muyanja, C.M.B.K., Narvhus J.A., Treimo J. and Langsrud T. 2003. Isolation, characterisation and identification of lactic acid bacteria from bushera: a Ugandan traditional fermented beverage. *International Journal of Food Microbiology* 80 (3): 201-210.
- Sanni, A.I. 1993 .The need for process optimization of African fermented foods and beverages. *International Journal of Food Microbiology* 18: 85–95.
- Sawadogo-Lingani H., Lei V., Diawara B., Nielsen, D.S., Møller P.L., Traore´ A.S. and Jakobsen M. 2007. The biodiversity of predominant lactic acid bacteria in dolo and *pito* wort for the production of sorghum beer. *Journal of Applied Microbiology* 103: 765–777.
- Schwartz M. 2001. Refractometer Conversion Table (Brix, Plato, S.G.), Zymurgy.
- Sefa-Dedeh, S. and Asante, S. 1988. Traditional alcoholic beverage from cereal in Ghana: processing of *pito* and burukutu in the Greater Accra Region, Ghana. KIFP Food System Research Report No. 1, p. 39.
- Sefa-Dedeh, S., Sanni, A. I., Tetteh, G. and Sakyi-Dawson, E. 1999. Yeasts in the traditional brewing of *pito* in Ghana. *World Journal of Microbiology and Biotechnology* 15 (5): 593-595.
- Shimizu, H., Mizuno, S., Hiroshima, T. and Shioya S., 2002. Effect of carbon and nitrogen additions on consumption activity of apparent extract of yeast cells in a brewing process. *Journal of American Society of Brewing Chemists* 60: 163–169.
- Villicana, M.T.O. and Saldivar, S.O.S. 2004. Production of Lager beer from sorghum malt and waxy grits. *Journal of American Society of Brewing Chemists* 62 (4): 140-146.