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Biotechnological Production of Non-Traditional Beer

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Abstract. In the present study we brewed sorghum (pito) and low-alcoholic beer (LAB) utilizing *Sacharomyces cerevisiae*, *Lactobacillus plantarum* and *Saccharomycodes ludwigii* as starters, respectively, and characterized their quality parameters. Single infusion method of mashing was practiced. Physiochemical, sensory and antiradical properties of samples were determined. Pito was produced by pitching wort with *S. cerevisae* (single starter culture (SSC)) and *S. cerevisae* in combination with *L. plantarum* (mixed starter culture (MSC)). ºBrix did not change over the next 24 hours for both cultures and began to decline, yet still it remain steady when the fermentation was over. After the end of fermentation, wort pitched with SSC showed lower ºBrix (6.63±0.11), than the wort with MSC (6.73±0.20) and differ significantly ($P < 0.05$) with duration of the fermentation process. LAB also exhibited a decrease in ºBrix from 12.2±0.12 to 8.04±0.01 at the end of the fermentation. Titratable acidity (TA) and pH remained constant after 24 hours of fermentation. TA began to increase from 0.73±0.02 to 1.04±0.02 and 0.73±0.02 to 1.07±0.02 for SSC and MSC, respectively. A decrease in pH from 4.33±0.20 to 3.86±0.15 and 4.33±0.20 to 4.2±0.1 was observed for SSC and MSC, respectively, during the rest of the fermentation period. A total of 22 volatile compounds including 11 esters, 3 alcohols and 8 acids, were identified in pito. Seven of these compounds were detected after the first fermentation (in green beer), whilst the rest (16 compounds) were distinguish after secondary fermentation. We also identified 8 volatiles in LAB, including 5 alcohols, 2 esters and 1 acid. Electron paramagnetic resonance spectroscopy of free radicals was used to determine the antiradical activity (AOA) of LAB in comparison with industrial alcoholic beverages (Baltica 7 from St Petersburg, Russia and Nectar beer from Bosnia-Herzegonia). LAB showed DPPH radical scavenging activity of 1.16 $\times 10^{-4}$ mol × equ (R^2=0.86) though Nectar beer exhibited the higher AOA of 1.17 $\times 10^{-4}$ mol x equ (R^2=0.69) whilst the least was Baltica beer 9.85×10^{-5} mol x equ (R^2=0.96). Panellists generally accepted the pito brewed with SSC (4.28±0.95) as well as LAB (3.85±0.69). All the parameters of beer assessed for the sensory evaluation were satisfactory.

INTRODUCTION

According to [1] beer is an alcoholic beverage made from malt, yeast, water, and hops. Sorghum beer, also known as pito, is a traditional local alcoholic beverage brewed from malted Sorghum. This type of beer is common throughout West Africa. It is one of the most recognized locally made beer in Ghana and it is produced in the northern part of Ghana, Upper East and West regions because of the abundant cultivation of sorghum in those mentioned regions. The color varies from golden yellow to dark brown with taste slightly sweet to very sour. It contains lactic acid, sugars, amino acids, 2–3 % alcohol and some vitamins and proteins [2].

Traditionally, fermentation of this beverage is uncontrolled and the microorganisms that intervene come from the raw materials, equipment and local environments or from residues of previous fermentation batch. These microorganisms, by virtue of their metabolic activities, play vigorous role in physical, nutritional and organoleptic modification of starting [3]. However, the wide variety of microorganisms present in a spontaneously fermented food gives product with widely varying quality [4].

Starter cultures were proposed as a suitable approach to improve the African traditional fermented food [5, 6]. The use of suitable starter improves the fermentation process, facilitates the control over the initial phase of fermentation and the predictability of derivatives products [6, 7].

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The starter cultures also reduce the organoleptic variations and the microbiological instability of African fermented foods [5]. The predominant microorganisms in African opaque beers (pito) are *Saccharomyces cerevisiae* and Lactic acid bacteria (LAB) [8–10]. Difficulties have been mention on the use of sorghum malt in brewing western beer dues to several inherent difference raw material characteristic [11]. The problem of lack of β-amylase in sorghum malt could be solved when sorghum is brewed in association with other crops available in Africa like millet other than using conventional enzymes [12].

The sale of beers with low alcohol content is a fast growing segment of beer market and is the focus for a lot of innovations therefore beer industries are facing major challenges. Also the creation of beers with low alcohol content had different historical reasons in the past. For example, during the World Wars (1914–1918 and 1939–1945) the shortage of raw materials led to the production of beers with low original extract (often with a high proportion of adjuncts) and thus of low alcohol content. Also the ban on production, sale and consumption of alcohol in United States of American during 1919 and 1933 is another reason [13, 14]. The main efforts on the production of low or alcohol free beverages are related to the final quality of products in terms of taste, aroma and flavour, which should be preserved or added to the product in order to obtain a beverage as similar as possible to the alcoholic one [15]. Impairment by alcohol is an important factor influencing both the risk of a road crash as well as the severity of the injuries that result from crashes [16]. Alcohol drinking has been associated with primary liver cancer, although this relation is difficult to investigate in epidemiological studies, since most alcohol-related liver cancers follow a cirrhosis, which leads to a reduction of alcohol drinking [17, 18]. Because of the problems stated above consumers have begun to demand for LAB, also due to health and social benefits resulting from LAB consumption, has given rise to the development of alcohol free beer. Drinking low alcohol beer is shown to be, at least, as effective as wine drinking at reducing risks of coronary diseases, heart attack, diabetes, overall mortality, obesity, metabolic syndrome, Parkinson's disease, physical health, Gallstones and ageing [19–27]. Therefore, the aim of the present study is to brew pito and LAB utilizing *S. cerevisae*, *L. plantarum*, *S. ludwigii* as starters and to characterize their quality parameters.

METHODOLOGY

Malting and Wort Production

The white sorghum and millet grains were obtained from the local market, Yekaterinburg, Russia. The grains were sorted manually to remove broken kernels and debris and then used for malting. The grains selected for malting were then steeped in distilled water (10 L) at 25˚C for 24 h. The grains were germinated at 30˚C for 4 days and then kilned by sun drying for 3 days. The shoots and rootlets were removed manually and the malt kernels were ground in a grinding mill. The wort was produced by infusion mashing procedure developed for sorghum [16]. 6.9 kg of milled malt (70 % sorghum and 30 % millet) and malted barley (3 kg) were mixed with hot water heated at 53˚C for 30 min to gelatinize malt starch. The flowchart of sorghum beer production process is presented in the Fig. 1. After cooling, the mixture was brewed according to the following mashing program: 63˚C for 60 min, 75˚C for 90 min and cooled to 30˚C. Rice husk was to enhance filtration, the filtrate was heated until boiling for 1 hour. Hops (Dr. Rudi) was added 10 min before the end of boiling time.

Preparation of Starter Cultures and Fermentation of Sorghum Beer

The yeast strain (*Saccharomyces cerevisae*) and lactic acid bacteria strain (*Lactobacillius plantarum*) used as starter cultures were obtained from Brewferm, Belgium and the Department of Technology for Organic Synthesis, Ural Federal University, Russia, respectively. *S. cerevisae* strain was sub-cultured on yeast extract dextrose peptone agar (YEPD) at 30˚C for 48 hours and then by successive sub-culturing on YEPD broth at 30˚C for 24 hours and 18 hours, respectively. *L. plantarum* strain culture was also sub-cultured at 37˚C for 48 h on cabbage extract medium (40 g cabbage, 4 g agar, 2 g peptone, 2 g $CaCO_3$, 4 g glucose, 200 ml tap water, pH=7.7) followed by two successive rounds of sub-culturing in cabbage extract broth (200 g cabbage, 10 g peptone, 10 g $CaCO_3$, 20 g glucose, 800 ml tap water, pH=7.7) with incubation at 37˚C for 24 hours and 16 hours, respectively. Yeast and LAB strains were each harvested by centrifugation at 4000 × g for 20 min and pellets were added in 50 ml of sterile sorghum worts and then incubated at 30˚C for 24 h in order to initiate fermentation. 10 L of sterile sorghum wort was transferred into two fermenters (20 L, GL-70/20, China) equipped with airlock bubbler and pitched with *S. cerevisae* (single starter culture (SSC)) and *S. cerevisae* in combination with *L. plantarum* (mixed starter culture (MSC)) followed by

the incubation at 18˚C for 96 h. Stainless steel keg was used for the carbonation after the primary fermentation. The 96 h old green beer was siphoned into the keg and pumped 1.8 bar of carbon dioxide (CO_2) from CO_2 tank fitted with automatic gas pressure regulator. It was then stored at 4°C for 1 week.

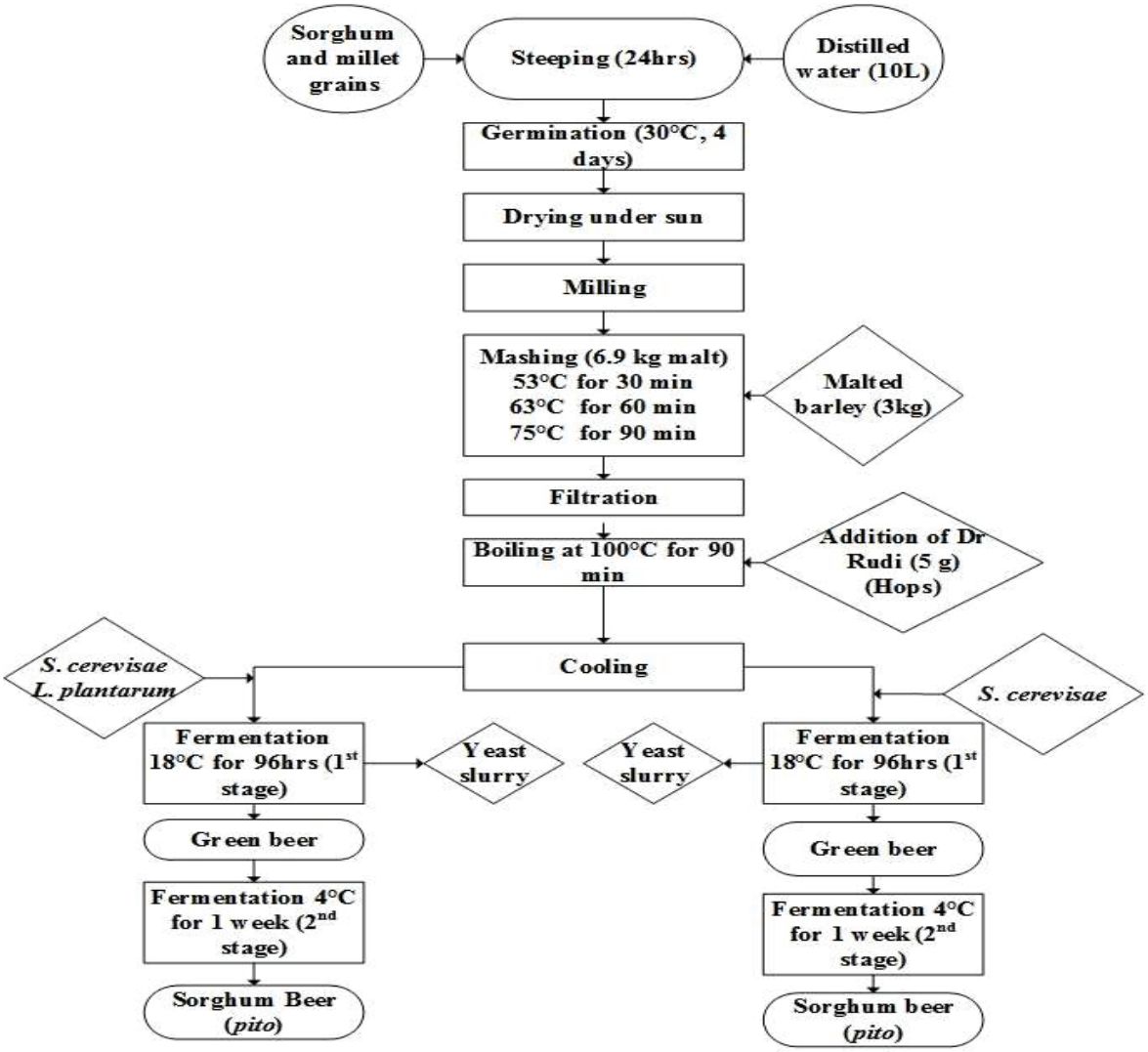


FIGURE 1. Flowchart of sorghum beer (pito)

PREPARATION OF *SACCHAROMYCODES LUDWIGII*

Saccharomycodes ludwigii (IHEM LY2014-0962) was purchased from Belgium Co-ordinated collection of microorganisms (BCCM-IHEM) of the Scientific Institute of Public Health-Section mycology and aerobiology, Belgium and maintained on Yeast Mold (YM) agar (3 g of yeast extract, 3 g of malt extract, 5 g of peptone, 10 g of dextrose, 20 g of agar) plates. S media purchase as slant along with the *S. ludwigii* was also inoculated to check the prepared YM agar. YM broth (3 g of yeast extract, 3 g of malt extract, 5 g of peptone, 10 g of dextrose) was also prepared. All the media and the glass wares was sterilised at 121˚C for 15 min. Suspension was made by picking colonies of *S. ludwigii* into sterilize beaker with sterile distilled water (5 ml) with the aid of sterilise loop. Pipette was then use to uniformly mix the suspension and divide into 2 ml each. 10 ml of YM broth was measured into 2 sterile Erlenmeyer Flasks each inoculated with the 2 ml *S. ludwigii* suspension. The mixture was then set on

mechanical shaker for 72 hrs at 150 rpm (24 ˚C). Viability of the cells was checked using hemocytometer. 10 ml of the mature culture suspension was then transferred into 2 Erlenmeyer flasks each containing 100 ml of fresh YM broth. The flasks were again set on mechanical shaker for 71 hrs at 150 rpm (24 ˚C). YM broth with *S. ludwigii* was then centrifuge to separate the supernatant from the biomass (cells). Hemocytometer was used to quantify the cells.

Production of Wort for Low Alcoholic Beer

Vienna malt, Fructose, liberty hops were used in brewing the low alcoholic beer (LAB). All these materials were purchased from BeersFan brewery in Yekaterinburg, Russia. The flowchart of LAB production process is presented in Fig. 2. Single infusion method was used by mixing 0.5 kg milled malt in 2 L distilled water in mash turn and heated up to 54°C for 20 min. The temperature was increased to 62°C with a step times of 15 min. The temperature was once again increased to 78°C after 45 min. Filtration was carried out, sparging was done using 17 ml distilled water. The filtrate was then boiled at 100°C for 90 min, liberty hops (2 g) was added 25 min before the end of the boiling. 30 g of fructose was also added to the wort before boiling. Wort was allowed to cool at desired temperature by placing the boiled wort under running cold water. The wort was then divided and transferred into two 500 ml Erlenmeyer flasks. With the aid of Hemocytometer, 1×10^9 cells/ml was pitched into the Erlenmeyer flasks containing wort and ferment at 25°C for 3 days.

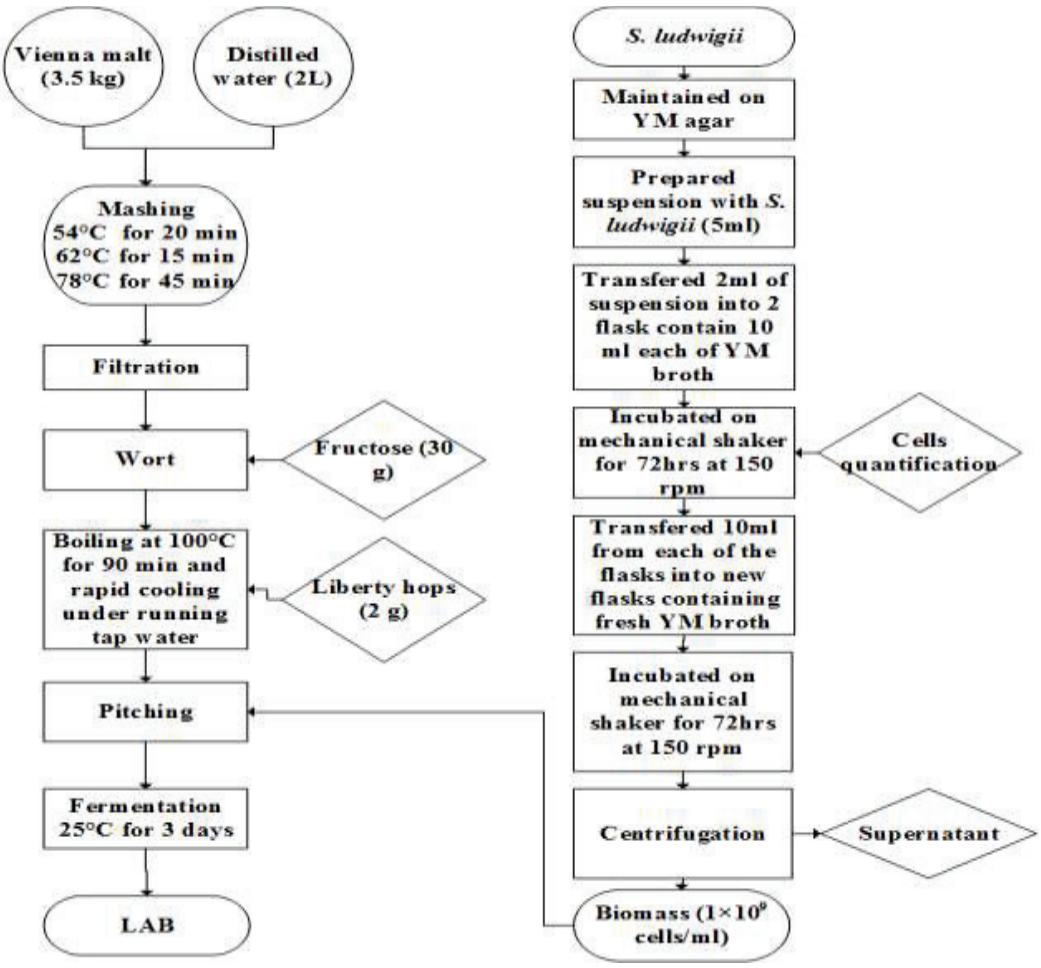


FIGURE 2. Flowchart of LAB production

Physicochemical Analysis

Refractometer was used to measure the °Brix. Sample of wort, dropped on the prism with the aid of sterilized pipette and the daylight plate place on it, reading was taken at the eyepiece. pH was determined using digital pH meter (Hanna, model HI 98127) by immersion into the wort and reading taken on the LCD screen. Total acidity was determined by titrating the samples with 0.1 N NaOH. The alcohol content of the sorghum beer and LAB in ABV, % was calculated according to the method described by [28] and pycnometer [29], respectively.

Volatile Compounds Analysis by Gas Chromatography–Mass Spectrometry (GC-MS)

GCMS-QP2010 Ultra, Shimadzu, gas chromatograph outfitted with mass spectrometer equipped with a capillary column DB-5 ms (30m x 0.25mm, 0.25 µm) covered with 5 % diphenyl-95 % dimethyl polysiloxane, was utilized. Helium was used as a carrier gas at a constant flow rate of 1.5mL/min. Without pre-treatment 1µL of the beer samples was injected in the splitless mode (vent time, 60 s) and the Compounds were identified by comparing of mass spectra obtained with Mass Spectral Library NIST.

DPPH Radical Scavenging Activity of LAB

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals scavenging activity was estimated by their Electron paramagnetic resonance (EPR) (Bruker Elexys E-500, X-band) spectra. The determination of antioxidant activity of beer samples was carried out by the difference in the amount of paramagnetic particles of a stable free radical, measured before and after complete proceeding the chemical reaction between a free radical and an analyte, which is accompanied by a decrease in the intensity of the EPR spectrum of the free radical after adding the analyte by the formula:

$$AOA = \frac{(n_{s1} - n_{s2}) \times C_{DPPH}}{n_{s1}} \quad (1)$$

where: AOA – the antioxidant activity (meq); C_{DPPH} – the DPPH concentration in an initial solution (M); n_{s1} – the initial amount of paramagnetic centers of DPPH, units; n_{s2} – the amount of paramagnetic centers of DPPH after interaction with the analyte (beers), units [30]. DPPH (1 mmol) was dissolved in ethanol and without pretreatment 10 µl of the LAB was pipetted into Eppendorf tube containing 1 ml of DPPH. EPR spectra of every 30 seconds for 15 minutes were recorded during the reaction with an antioxidant using EPR spectrometer Bruker Elexys E-500, X-band.

Assessment of Organoleptic Properties of Sorghum Beer and LAB

A total of 7 panellists consisting of unequal numbers of males and females of different ages were randomly selected from the Department of Technology for Organic Synthesis in the Institute of Chemical Engineering. Transparency (excellent – 3, good – 2, satisfactory – 1, remove from test – 0), flavour (excellent – 4, good – 3, satisfactory – 2, poor – 1), colour (excellent – 3, good – 2, satisfactory – 1, poor – 0), foaming and saturation with carbon dioxide (excellent – 5, good – 4, satisfactory – 3, poor – 2, remove from test – 0), taste (excellent – 5, good – 4, satisfactory – 3, poor – 2), hop bitterness (excellent – 5, good – 4, satisfactory – 3, poor – 2) and the overall acceptability (excellent – 5, good – 4, satisfactory – 3, poor – 2) of the beer samples were evaluated. The panellists were required to be regular consumers of beer.

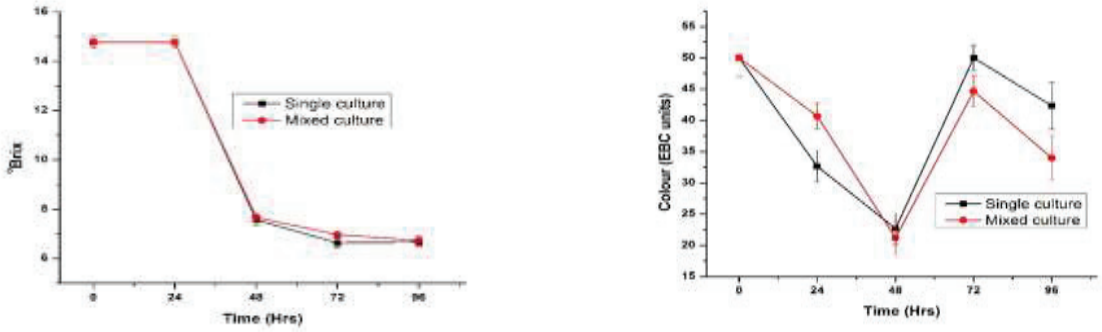
Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 8.1) at 5 % significance. All measurements were made at least in triplicate. Results were reported as means±standard deviations. For sensory analysis a one-way ANOVA and a post-hoc Tukey-Scheffe test were conducted to test the means results obtain at 5 % significance.

RESULTS AND DISCUSSION

Physiochemical Analysis

From Fig. 3(a) the °Brix did not change over the next 24 hours for both cultures and began to decline still it remain steady when the fermentation was over. After the end of fermentation, wort pitched with SSC registered lower brix (6.63±0.11) than that of the MSC (6.73±0.20) and differ significantly ($P < 0.05$) with duration of the fermentation process. °Brix gives an idea about the amount of sugars in the wort, and the decline in value means the starter cultures are utilizing them for it metabolism in other to produce alcohol [31]. The decrease in this characteristic from 14.7±0.25 to 6.66±0.15 and 14.7±0.25 to 6.73±0.20 within 96 hours of fermentation period for SSC and MSC, respectively, supported the statement (Fig. 3(a)). This was also explained in early research [28], as fermentation progresses, simple sugars were converted into ethanol and carbon dioxide, and consequently the solids content of the wort was reduced. There was no supplementary decline in °Brix observed after 96 hours. LAB also exhibited decrease in °Brix from 12.2±0.12 to 8.04±0.01 at the end of the fermentation (Table 1). *S. ludwigii* utilises sugars (glucose, sucrose, raffinose) but partially ferment them [32] hence producing beer with low alcohol unlike the conventional yeast which uses all sugars in the wort enabling it to produce beer with products with higher ethanol content.



(a) (b)

FIGURE 3. Kinetics of Brix (a) and colour (b) vs. fermentation time

TABLE 1. Physiochemical analysis of LAB.

Sample	Wort		LAB				
	pH	°Brix	pH	Ethanol (%ABV)	Titratable acid (%)	°Brix	Colour (EBC)
LAB	5.5±0.1	12.2±0.12	4.26±0.05	1.12	0.83±0.05	8.04±0.01	46.3±0.57

Wort colour began to change before the 24 hours of fermentation for both the starter cultures. MSC wort registered higher colour than the SSC before 48 hours of fermentation time and decline more than the SSC after the 48 hours. An increase was observed at 72 hours from 50±3 to 42.3±3.78 and 50±3 to 34±3.60 EBC units for SSC and MSC, respectively (Fig. 3(b)). The increase might be because the fermentation was intense at that particular time and resulting in foam formation and accumulation yeast metabolites. The results were in agreement with earlier report by [28], who also observed an increase in the colour at 24 hours of fermentation time. Duration of fermentation had significant ($P < 0.05$) effect on the colour. Colour of the final LAB in EBC was 46.3±0.57, which was higher than colour of conventional beer could be because of the partial fermentation of wort with *S. ludwigii* leaving sugars (galactose, maltose, lactose, melibiose and trehalose) [32] contributing to the colour of the final product.

At the beginning of the fermentation there was no alcohol formation in the wort this remained the steady till 24 hours. After 24 hours, starter cultures starts to utilise the sugars in wort resulting in alcohol formation until when the

fermentation was over after 96 hours (Fig. 4). SSC recorded higher alcohol (4.02±0.03 to 4.54±0.04 %ABV) than MSC (3.78±0.03 4.5±0.03 %ABV).

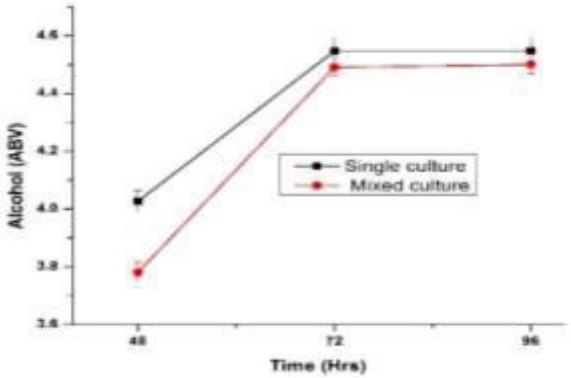
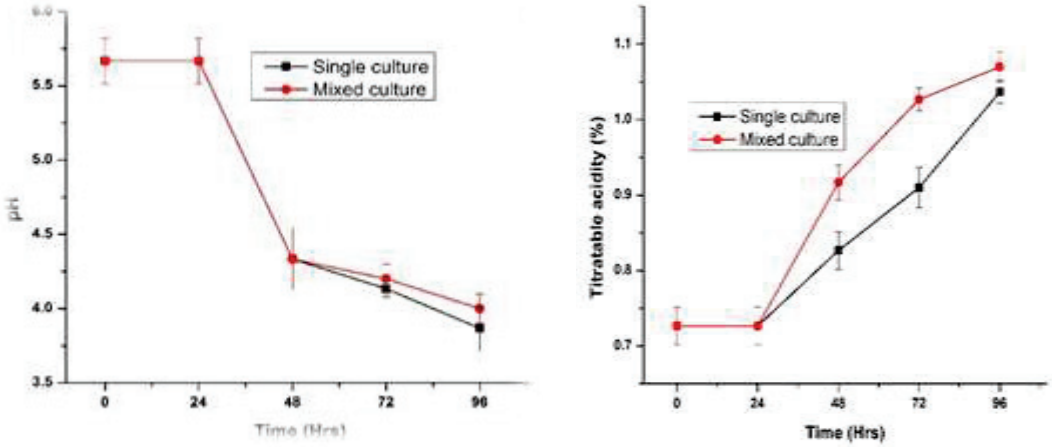


FIGURE 4. Kinetics of alcohol formation over fermentation period

Alcohol was not detected at the early stage (0–24 hrs) of the fermentation because starter cultures were still acclimatising in the new environment (wort) and could not consume the sugars. It was when they were now familiar with the wort that they began to use to sugars. The alcohol contents recorded were not in agreement with [28] who reported values to be from 1.1 to 2.78 %, probably dues to difference in strains of starter cultures and fermentation conditions. Duration of fermentation had significantly ($P < 0.05$) effect on the alcohol content of the pito. *S. ludwigii* is considered as reasonable yeasts to produce low alcohol beer [33, 34] due to its total or partial inability to ferment maltose, which is the principal fermentable sugar in wort [32]. The alcohol content of the beer was 1.18% (ABV) which is lower than when conventional yeast was used as a starter. Among 6 strains of *S. ludwigii* used by [32] in their studies, DBVPG 3931, DBVPG 4116 produced alcohol with the percentage of 1.24 and 1.36 (% v/v), respectively. The amount of sugar which yeast consumes correlated with the quantity of ethanol it produces and *S. ludwigii* utilised few sugars leaving the main sugars in the wort hence produced low amount of ethanol.

Titratable acidity (TA) and pH remained constant after 24 hours of fermentation. But TA began to increase (Fig. 5(b)) whilst the pH decrease (Fig. 5(a)) during the rest of the fermentation period. Wort pitched with MSC exhibited higher pH and TA in 72, 96 hours. TA increased right after 24 hrs of fermentation till the end of fermentation period.

A decrease in pH from 4.33±0.20 to 3.86±0.15 and 4.33±0.20 to 4.2±0.1 in 48 hours for SSC and MSC, respectively, was observed. The further decline was seen after 72 hours for both starters. This could be associated with the build-up of metabolites as a result of the fermentation and also at stress conditions for starter cultures. Moreover, organic acids including succinate, lactate, and acetate are products of the metabolism of brewing yeast and their secretion contributes to the characteristic drop in pH that occurs during fermentation [28]**.** The fermentation time and culture type, influenced on pH of the wort fermenting statistically ($P < 0.05$). No changes were observed in TA before 24 hours of fermentation but it began to increase from 0.73±0.02 to 1.04±0.02 and 0.73±0.02 to 1.07±0.02 for SSC and MSC, respectively, once fermentation progressed. It had significant effect on TA ($P < 0.05$). Similar results were reported by [35, 36], and [37] on pito and dolo. This trend was probably observed because of fermentation progress. Substrates (e.g. glucose) in wort are used up and metabolites began to be accumulated leading to increase in TA. pH of wort (LAB) decreased from 5.5±0.1 to 4.26±0.05 (Table 1) till the end of the fermentation. The initial pH of the wort was similar to the pH of the wort used by [32] in their study. The low pH (4.26±0.05) of LAB is good as this help to inhibit microorganism and prevent microbial staling of the product. TA of the LAB is 0.83±0.05, the conversion of wort to LAB is followed by decline in pH, this affect TA of the beer [31]. As pH decrease, acids are accumulated which cause a rise of TA of the beer.



(a) (b)

FIGURE 5. Kinetics of pH (a) and titratable acidity (b) *vs*. fermentation

Volatile Analysis with GC-MS

GC-MS analysis of pito and LAB for volatile compounds identification produced with cultures (SSC, MSC) and S. ludwigii, respectively, are outlined in Table 2 and 3. A total of 22 compounds were identified in pito including 11 esters, 3 alcohols and 8 acids. Seven of these compounds were detected after the first fermentation (in green beer), whilst the rest (16 compounds) were distinguished after the two weeks' maturation process i.e secondary fermentation (Table 2). Seven compounds (ethyl decanoate, isobutyl acetate, beta-methylproyl ethanoate, 2-methylbutyl acetate, 2- methyl-1-butyl acetate and dodecacanoic acid) were not detected in pito produced by SSC after the maturation process while two compounds were absent in pito brewed with MSC (-propan-1-ol and 3-methyl acetate). In LAB, 8 volatiles were identified including 4 alcohols, 2 esters and 1 acid (Table 2). The volatile identified in the present study were compared with literature and it was found that, 5 among the 8 were detected in other works [32, 34, 38].

According to [31] esters are produced during a vigorous phase of the first fermentation by the enzymatic chemical condensation of acids and alcohols. Ester (Ethyl Acetate), acid (acetic acid) and alcohol (2-methylpropan-1-ol, 3-methylbutan-1-ol) were the abundant compounds in the pito produced by both cultures before and after maturation processes. Esters are accountable for the fruity-flowery aromas in fermented drinks and were detected in many fermented beverages [4, 39–41]. Only 2 esters were identified in the present study which might be as a result of the lack of essential enzymes which S. ludwigii is produced for the transformation of acid and alcohols to esters. Also the inability of the yeast to ferment all sugars in wort might be a reason. In a previous study, strains of S. ludwigii produced different amount (mg/L) of esters (DBVPG 3010, 14.91; DBVPG 3054, 2.06; DBVPG 3304, 1.56; DBVPG 3398, 1.21; DBVPG 3931, 2.23; DBVPG 4116, 4.15) [32]. It supported the fact of formation of 2 esters in the present study.

Higher alcohols (HA) are compounds that have more carbon atoms than ethanol [42] and contribute to beer flavour due to their alcoholic or solvent- like aroma causing a warm mouth- feel [43]. The sugars, free amino acids, yeast, and temperature affected the formation of the higher alcohols. These alcohols contributed to flavour in beer and other beverages [28]. Propan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol were identified in pito brewed in the presence of two cultures, whilst propan-1-ol was detected in pito obtained from SSC after second fermentation. A total of 5 alcohols were identified in LAB and 3 were found in previous work by [32, 34, 38]. The efficient uptake and utilization of amino acid and sugar determined the final quantity of higher alcohols in beer.

Organic acids (OA) in beer are derived from wort and yeast metabolic cycles. The short carbon skeleton organic acids secreted by yeasts are derived from the incomplete turnover of citric acid cycle and the amino acids catabolism. OA truncates the pH during fermentation conferring a sour taste. It also provides an increase of microbial balance on the final product [41, 44]. Acetic acid was detected in all the pito samples after the first fermentation whilst the other acids were only identified after the second fermentation of the green pito obtain from both cultures. According to [28] the by-product of metabolism of amino acid and sugar by starters produced alcohols, aldehydes, organic and fatty acids and esters of alcohols and fatty acids which are the principal flours in

alcoholic beverages. Similar results were obtained in [4]. Acetic acid was the only acid identified in LAB. In previous works [34, 38, 32] acetic acid was not detected among the volatiles compound identified, this might be as a result of different strains, mashing temperature regimes and fermentation conditions.

TABLE 2. Volatile compounds obtained by fermenting pito with SSC, MSC and comparing with literature sources.

Compounds	Primary Fermentation		Secondary Fermentation (Maturation)		[4]		[28]	
	SSC	MSC	SSC	MSC	SSC	MSC	SSC	MSC
Esters								
Ethyl Acetate	+	+	+	+	+	+	+	+
3-methyl-acetate	+	+	+	*	*	*	*	*
Ethyl butanoate	*	*	+	+	*	*	*	*
Ethyl hexanoate	*	*	+	+	*	*	*	*
Ethyl caproate	*	*	+	+	+	N.D	N.D	+
Ethyl decanoate	*	*	*	+	*	*	*	*
Ethyl octanoate	*	*	+	+	*	*	*	*
Isobutyl acetate	*	*	*	+	+	+	*	*
2-Methyl-1-butyl acetate	*	*	*	+	*	*	*	*
2-Methylbutyl acetate	*	*	*	+	+	N.D	*	*
Beta-methylpropyl ethanoate	*	*	*	+	*	*	*	*
Alcohols								
Propan-1-ol	*	*	+	*	+	+	*	*
2-methylpropan-1-ol	+	+	+	+	+	+	*	*
3-Methylbutan-1-ol	+	+	+	+	+	+	*	*
Acids								
Acetic acid	+	+	+	+	+	+	*	*
Butanoic acid	*	*	+	+	N.D	+	+	+
Hexanoic acid	*	*	+	+	*	*	*	*
Propanoic acid	*	*	+	+	+	N.D	*	*
Octanoic acid	*	*	+	+	*	*	*	*
Caproic acid	*	*	+	+	N.D	+	*	*
Dodecanoic acid	*	*	*	+	*	*	*	*
Isobutyric acid	*	*	+	+	*	*	*	*

ND, not detectable**, (**+) indicate present and (*) indicate absent of volatile compound

TABLE 3. Volatile compounds identified by fermentation with *S. ludwigii* comparing with literature data.

Volatile Compounds	[34]	[38]	[32]
Ethanol	+	+	+
Ethyl Acetate	+	+	+
Acetic acid	*	*	*
2-methylPropan-1-ol	*	*	*
3-methylbutan-1-ol	*	*	*
2-methylbutan-1-ol	+	+	+
Isoamyl acetate	N.D	+	+
Isobutanol	+	+	+

ND, not detectable, (+) indicate present and (*) indicate absent of volatile compound

In pito beer, maturation assisted to the development of the additional volatile compounds. This step is normally excluded when the pito is brewed by the Ghanaian locals because the product is served to customers after the first fermentation due to it short shelf life.

ORGANOLEPTIC PROPERTIES ASSESSMENT

The pito obtained from SSC and LAB were evaluated for consumer acceptability using 7 panellists who were regular consumer's western beers. Pito obtained with mixed culture was removed from the test because it did not meet the criteria specified by the questionnaire use for the sensory analysis.

TABLE 4. Scores of organoleptic properties of Pito fermented with SSC and LAB produced by *S. ludgiwii*.

Sample	Transparency	Colour	Flavour	Taste	Hops Bitterness	Foaminess	Overall Acceptance
SC	1.14±0.37	2.57±0.53	3.42±0.78	4.28±0.75	4.14±0.89	4.42±0.534	4.28±0.95
LAB	2.71±0.48	2.14±0.89	3±1	3.57±0.78	2.71±0.48	2.85±1.06	3.85±0.69

SC-single culture pito, LAB-Low alcohol beer

According to [45] sensory evaluation can be defined as a scientific discipline used to evoke, measure, analyse and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing. All the panellists except one indicated satisfactory for transparency of the beer (1.14±0.37). Transparency in beer has correlation with the filtration during the wort production, as poor filtration will result in beer which will not be transparent. The assessors scored 2.71±0.48 for the LAB.

The production of volatile compounds is a result of enzymatic cycles (to produce end-products like succinate, acetate, formate, lactate) which are attributed to the flavour of the pito. Four panellists perceived the flavour as excellent, two as good while one person gave satisfactory evaluation. By comparing the flavour scores (3.42±0.78) with the volatile compounds detected in the present study, it was found that esters and alcohols contributed to the flavour. Dual hops were used in wort boiling which contributed to flavour of the pito. The mean score for LAB flavour is 3±1 which is quite good base on the panallist responses. Liberty and aroma hops were utilised in producing the LAB which might contribute to the flavour of the LAB.

The average mean for taste (4.28±0.75) and hop bitterness (4.14±0.89) of pito scored between good, satisfactory and excellent which indicate that the yeast utilized majority of the sugars during the fermentation which balance the sweet taste of the beer with the bitterness originating from the hops. Initially, wort was sweet because of the degradation of starches in the grains to various sugars (maltose, fructose etc) during mashing possess using different temperature regimes by action of enzymes. As fermentation progress yeast consume these sugars resulting in alcohol formation leading to diminishing of sweet taste. The taste of LAB 3.57±0.78 was slightly sweet base on response we got from the assessor and this might be as a result of *S. ludwigii* inability to completely utilise the wort sugars. The remaining sugars contributed to the sweet taste the assessors claimed.

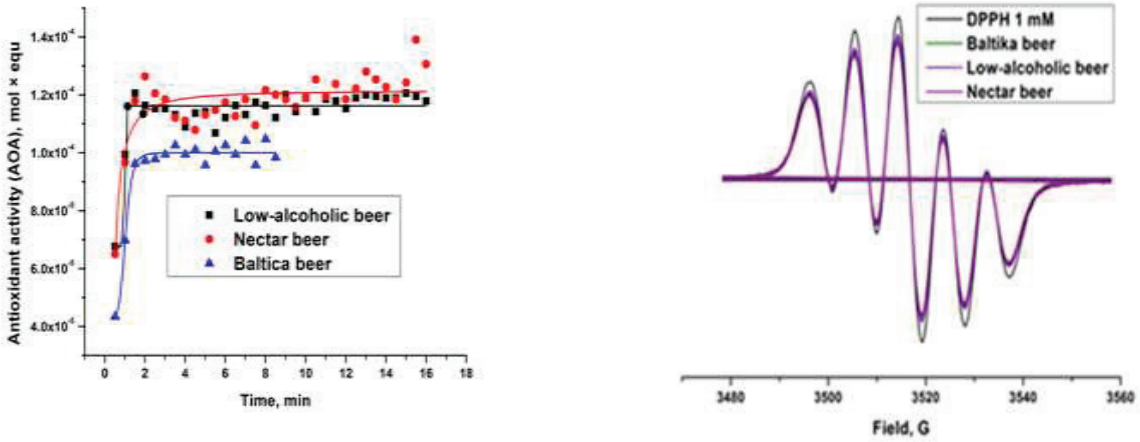
The average scores for foaminess 4.42±0.53 were good. It indicated that the carbonation step was efficiently done to replace the lost CO_2 from the pito. Consumers might regard beer without foam when pouring as juice and might not patronise these sort of beer. The assessor also liked LAB foaminess (2.85±1.06), though Carbonation step was not practice, this could have aid the foaminess of the LAB. The acceptability of the beer produced from SSC was impressive judging from the panellists scores (4.28±0.95). LAB was likewise accepted by the assessor based on the average mean of the scores 3.85±0.69.

ANTIRADICAL ACTIVITY (ADA) OF LOW ALCOHOL BEER

Flavan-3-ols derivatives, flavon glycosides and phenolic acids are the main phenolic compounds that originated from barley into beer. These compounds are described to have anticancer effect due to their powerful antioxidant activities [46, 47, 48, 49]. EPR spectroscopy of free radicals was used to determine AOA of LAB in comparison with industrial alcoholic beverages (Baltica from St Petersburg, Russia and Nectar beer from Bosnia). The kinetics and spectra of DPPH radical interacting with the beverages under study are shown in Fig. 6(a) and 6(b). LAB

showed DPPH radical scavenging activity of 1.16 $\times 10^{-4}$ mol × equ (R^2=0.86) though Nectar beer exhibited the higher AOA of 1.17 $\times 10^{-4}$ mol × equ (R^2=0.69). The least activity was demonstrated by Baltica beer, 9.85×10^{-5} mol × equ (R^2=0.96). From Fig.8 it can be seen that the difference in AOA of LAB and Nectar beer is not quite significant. The malt, hops and *S. ludwigii* used in brewing the LAB might have contributed phenolic compounds which inhibits free radicals.

Among the numerous antioxidants in beer, polyphenols are of interest to brewers as they play a vital role in inhibiting the oxidation process through prevention of free radical scavenging and metal chelation which helps physical and flavour stability of the beer. Polyphenols levels in beer are associated with the type of malting, brewing techniques and materials (hops and malts varieties) [1]. *S. ludwigii* requires nutrient for efficient conversion of sugar to LAB and sulfite is one of the vital nutrients which the yeast obtain by converting sulfate. It has been underline to be one of the productive antioxidant in LAB [50].



(a) (b)

FIGURE 6. DPPH radical scavenging activities (a) and EPR spectra (b) of samples *vs*. time

CONCLUSIONS

The present study provided with information on the use of *S. cerevisiae*, *L. plantarum*, *S. ludwigii* as starter cultures for the production of Ghanaian traditional sorghum beers and low alcoholic beer, respectively. The SSC and MSC exhibited similar feature during the fermentation period. We were able to produce LAB with alcohol percentage of 1.12 (%ABV). The association of *S. cerevisiae* and *L. plantarum* aided the development of volatile compounds but did have effect on the taste. A total of 22 and 8 volatiles were identified in pito and LAB respectively. LAB had slightly sweet taste. It is recommended to use S. cerevisiae alone as starter culture to produce pito beer having the good organoleptic characteristics. This study also contradicts previous works [4, 28]. Panellists generally had a higher preference for pito beer obtained from SSC and LAB. Antiradical activity of LAB was good since it will help to prevent cancer and stabilise the beer itself.

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