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Research Paper

Influence of yeasts on bioactive compounds content of traditional sorghum beer (tchapalo) produced in Côte d'Ivoire

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ABSTRACT

Traditional sorghum beer is reputed for its therapeutic virtues in according the consumers. A number of biological active compounds like phenolic compounds (phenol, tannins, flavonoids, anthocyanins), diets fibers and compounds with clinically demonstrated antimalarial activity (quinine formate, quinine dihydrochloride, chloroquine) and antioxidant activity (2,2-diphenyl-1-picryl-hydrazyl and ferric reducing-antioxidant power methods) were evaluated in sorghum wort and beers fermented by wild yeasts and pure culture of *Saccharomyces cerevisiae*. The total phenol content in the samples ranged between 1254.69 ± 2.31 and 239.68 ± 11.92 μg/mL GAE. Antioxidant activity with 2,2-diphenyl-1-picryl-hydrazyl analysis method was high in sorghum wort with 73.33 ± 1.15% but with ferric reducing-antioxidant power analysis method, the antioxidant activity was high in beer from pure culture of *Saccharomyces cerevisiae*. No compounds with clinically demonstrated antimalarial activity were found in the samples. At bioactive compounds (phenolic compounds) content point view, statistical analysis showed similarity between the two beers.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) plays a crucial role in food security in developing countries. It is involved in cooking of many foods such as breads, porridges, pastes and pancakes. It is also abundantly used to prepare traditional beers commonly named sorghum beers or opaque beers but known as pito or burukutu in Nigeria, chibuki in Zimbabwe, dolo in Mali and Burkina Faso, bili bili in Chad and tchapalo in Côte d"Ivoire (Maoura et al., 2005; N'Guessan et al., 2010). The processing of African sorghum beer involves malting, drying, milling, souring (lactic acid fermentation), boiling, mashing, alcoholic fermentation and straining (Haggblade et al., 1989; Maoura et al., 2006; Sawadogo-Lingani et al., 2007), in which variations may occur depending on the regional location (van der Aa Kühle et al., 2001). Although production was originally limited to the Northern part of the country (Yao et al., 1995), today tchapalo can be found in any city with commercial activity. This expansion may be partially attributed to the migration of populations from the North of Côte d'Ivoire to the other regions, but the therapeutic characteristics (laxative and antimalarial properties) attributed to it by consumers might also play a role. Since it has been shown that sorghum (*Sorghum bicolor*), contains large quantities of phenolic compounds with considerable antioxidant activity (Devi et al., 2011), it may be assumed that at least part of the alleged therapeutic effects of sorghum beer can be attributed to these compounds. The sorghum has various applications in African traditional medicine and indeed, the traditional medicine uses of sorghum have been mentioned in literature. In Lagos state, Nigeria, sorghum leaf is used in local herbal medicine in an infusion of sorghum leaf with sliced *Randia lucida* roots that have been soaked in potash water is used as abortifacient, and sorghum leaf in a mixture with *Xylopia aethiopica* fruit, and *Afromomum melegueta* seeds in hot lemon juice is drank as a contraceptive (Balole et al., 2009). Again, in Lagos, Nigeria, sorghum is also used for the treatment of anemia, pain and inflammation. In South Western Nigeria, sorghum is employed in the treatment of headache, sickle-cell anemia, leukemia, multiple myeloma, and heart and

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blood-related problems. In India, sorghum is used as an aphrodisiac. A decoction of sorghum grains is demulcent and diuretic and is used for treating kidney and urinary tract complaints. The red pigment from sorghum is said to have antimicrobial and antifungal properties and is also used as a cure for anemia in traditional medicine (Lim, 2013).

Due to the ethno-pharmacological importance of traditional sorghum beer (tchapalo), the present study was designed to assess its phenolic compounds, diet fibers and antioxidant activity. For this, phenolic compounds, diet fibers and compounds with antimalarial properties were quantified and their antioxidant activity was measured. The sorghum wort and traditional sorghum beers contained an important amount of phenolic compounds but none compound with antimalarial properties.

2. Materials and methods

2.1. Yeast strains and sorghum wort

The yeast strain used in this study was *S. cerevisiae* F12-7. This strain belonged to the culture collection of the Food Technology Department (University Nangui Abrogoua, Abidjan, Côte d'Ivoire). This strain was isolated from traditional sorghum beer from the district of Abidjan (Southern Côte d'Ivoire). It was identified by Polymerase Chain Reaction-Restriction Fragment Length polymorphism (PCR-RFLP) of the Internal Transcribed Spacer (ITS) region and sequencing of D1/D2 domains of the 26S rRNA gene (N'guessan et al., 2011). *S. cerevisiae* F12-7 has been freeze-dried as described by Coulibaly et al. (2016).

The wild yeasts (traditional inoculum) which were harvested from previous brews and sun dried and sorghum wort were obtained from randomly identified commercial tchapalo brewers at Cocody-Anono in the District of Abidjan (Southern Côte d'Ivoire). The sorghum wort was sampled according the method described by Aka et al. (2008).

2.2. Fermentation experiment

Fermentations were carried out in triplicate under agitation in 1-L sterile Erlenmeyer flasks, fitted with dense cotton plugs and, containing 500 mL of pasteurized (10 min at 100 °C) sorghum wort. Five (05) grams of the freeze-dried *S. cerevisiae* strain and five (05) grams of wild yeasts were separately pre-wetted in 100 mL of sterile sorghum wort for 30 min at 30 °C before inoculation. Flasks were simultaneously inoculated with pre-culture to obtain an inoculation rate of 1% and incubated for 24 h at 28 °C with stirring speed to 150 rpm. From the sorghum wort, two beers were produced, beer from pure culture of *S. cerevisiae* (BPC) and beer from wild yeasts (BWY).

2.3. Physicochemical analysis of sorghum wort and beers

2.3.1. pH, titratable acidity and density

Value of the pH was determined with a pH-meter (Hanna Instruments; HI 8010) after calibration with KCl buffer. Titratable acidity was determined through titration with 0.lN NaOH. The titratable acidity was expressed as % meq of lactic acid. Density of each beer was determined by using of densimeter (Mettler Toledo). Two independent measurements were made on each sample.

2.3.2. Determination of sugars and ethanol content in the sorghum wort and beers (BPC and BWY)

The total soluble solids (TSS) content, expressed as °Brix, were determined in each sample using hand refractometer. Water-soluble carbohydrates were determined by the phenol sulfuric acid method, according to Dubois et al. (1956) and total reducing sugars were quantified using the dinitrosalicilic acid method described by Bernfeld (Bernfeld and Colowick N.O.K, 1955). Water-soluble carbohydrates and total reducing sugars contents were used to determine the saccharification rate. Ethanol was quantified by HPLC (Agilent Technologies, 1200 series, UK) using a column Aminex HPX-87H, 300 mm 7.8 mm (Biorad) coupled to a refractometer (Agilent Technologies) and a UV diode array detector (Agilent Technologies).

2.4. Phytochemical analysis of sorghum wort and sorghum beers

2.4.1. Total phenols

Total phenols contents were determined according to the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). To 50 μL aliquot of final product after centrifugation were added 250 μL of diluted Folin-Ciocalteu-reagent (10% v/v). After 1 min, 750 μL of 20% (w/v) aqueous Na_2CO_3 were added, and the volume was adjusted to 5.0 mL with H_2O. The controls contained all the reaction reagents except the sample. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm and compared to both a gallic acid calibration curve and to controls. Total phenols were determined as gallic acid equivalents per mL (μg GAE/mL), and the values were presented as means of triplicate analyses.

2.4.2. Total tannins

The determination of easily extractable tannins was carried out using the Bate-Smith reaction, in which colorless proanthocyanidines are converted into colored anthocyanins through heating at 100 °C in acidic medium and their levels measured based on their absorbance at 550 nm (Ribereau-Gayon and Stonestreet, 1966). In two test tubes, 2 mL of sample, 1 mL of distilled water and 3 mL of 12 N hydrochloric acid were added. One of the sample test tubes was left standing while the other was hermetically sealed placed in water bath at 100 °C for 30 min, and then cooled for 10 min in ice. Ethanol, 0.5 mL was added to each of the test tubes and their optical densities measured. The concentration of tannins, which is proportional to the concentration of anthocyanins, was calculated in g/L using the following equation (1):

$$\text{Tannins} = 19.33 \times \Delta\ \text{OD} \quad (1)$$

Where ΔOD is the variation in optical density between the two tubes.

2.4.3. Total flavonoid contents

Total flavonoid contents were determined a using the $AlCl_3$ colorimetric method (Meda et al., 2005). 0.5 mL of sample was mixed with the same volumes of distilled water, aluminum trichloride ($AlCl_3$) 10% (w/v) (Labosi, Paris, France), sodium acetate (1 M) and 2 mL of water. After 30 min of incubation at room temperature, absorbance at 415 nm was measured on a Rayleigh (UV spectrophotometer; USA). Total flavonoid levels were calculated from means of three replicates against a 0–300 μg/mL quercetin calibration curve (Sigma–Aldrich Chemie, Steinheim, Germany) and expressed as μg of quercetin equivalents (QE)/mL.

2.4.4. Total anthocyanins

Anthocyanins (At) are found in two forms: free anthocyanins (Af), which are susceptible to discoloration by SO_2, and anthocyanins combined with tannins (Ac), which are not (Ribereau-Gayon and Stonestreet, 1965). A mother solution was prepared by adding: 1 mL of sample, 1 mL of ethanol (95%) acidified with 0.1% hydrochloric acid (HCl) and 20 mL to 2% hydrochloric acid (35%). In a first test tube, 5 mL of the mother solution was added to 2 mL of distilled water. In a second test tube, 5 mL of the mother solution was added to 2 mL of sodium bisulfite (15%). After incubation of both test tubes for 20 min at room temperature, the absorbance at 520 nm was measured for an optical path of 10 mm. The concentration was calculated in mg/L by the following relation equation (2):

$$\text{Anthocyanins} = 875 \times \Delta\text{OD} \quad (2)$$

Where ΔOD is the variation of optical density between the first tube and the second tube.

2.5. Antioxidant activities

2.5.1. Antiradical activity: DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay

The DPPH assay method, one of the commonly used methods for the investigation of the antioxidant capacity of natural products, was used for its ease of use and accuracy (Alves et al., 2010). The antioxidant activities of beer and sorghum wort samples are determined by evaluating their respective free radicals scavenging abilities in the presence of an alcoholic solution of DPPH, yielding the free radical form DPPH°, Brannd-Williams et al. (Brand-Williams et al., 1995). The samples were mixed with the stable DPPH radical in a methanol solution. The reaction mixture consisted of adding 2 mL of sample, 2 mL of DPPH radical solution 100 mM in methanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced with a change. In color from deep violet to light yellow. The changes in color after 30 min of reaction time were read as Absorbance (Abs) at 517 nm using a Rayleigh UV spectrophotometer, USA. The rate of scavenging activity (AA%) was calculated as follows using equation (3):

$$(\%)\ AA = [(X–Y)/X] \times 100. \quad (3)$$

Where X is the absorbance at 517 nm of oxidized DPPH in pure unreacted form and Y is the absorbance of the sample after 30 min incubation with DPPH.

2.5.2. Ferric reducing-antioxidant power FRAP (potassium ferricyanide-ferric chloride) assay

The ferric ion (Fe^{3+}) -reducing capacity of beers and sweet wort samples was investigated by using the potassium ferricyanide-ferric chloride method (Liuk et al., 2009). 0.5 mL of each sample, 0.5 mL of phosphate buffer (0.2 M, pH 6.6), and 0.5 mL of potassium ferricyanide $K_3Fe(CN)_6$ (1%) were mixed and incubated at 50 °C for 20 min. The reaction was stopped by adding 0.5 mL trichloroacetic acid (10% (w/v)). 0.5 mL of the reaction mixture was mixed with 0.8 mL of distilled water and 0.1 mL of $FeCl_3$ (0.1%) and absorbance measured at 700 nm. The reducing power of each sample was expressed as μg of ascorbic acid equivalents (AAE) per mL.

2.6. Chemical analyses

2.6.1. Compounds with antimalarial properties

These compounds were determined by GC/MS method. The analytical column, a μBondapak Waters, C18, 10μ (300 × 3.9 mm), was operated at ambient temperature with backpressure of 230 kg/cm^2. The mobile phase consisted of heptane sulfonic acid 0.005 M/Acetonitrile, (66:34 %v/v) and was delivered at a flow rate of 1.0 mL/min. Fluorescence detection was performed at 344 nm (excitation) and 375 nm (emission). For the quantitative determination of chloroquine, quinine formate, quinine dihydrochloride (Sigma St. Louis, MO) was used as internal standard at a concentration of 0.5 ng/mL, resulting in a detection limit (signal-to-noise ratio 3:1) of 0.3 ng, while the upper limit of linear range was 0.7 ng/mL. Analysis time was less than 5 min. Values are presented as means of three measurements.

2.6.2. Diets fibers content

Determination of fibers content was performed according to the method of Wolf (Wolf and Manuel d'analyses d, 1968). An amount of 25 mL of traditional sorghum beer was introduced into a flask and homogenized with 50 mL of 0.25 N sulfuric acid. The mixture was heated to boiling with the aid of a heating cap (JP Selecta, Spain) and boiled for 30 min under reflux condenser. Then sodium hydroxide, 50 mL of 0.31 N were added to the contents and boiled again for 30 min under reflux condenser. The extract obtained was filtered on Whatman N°. 40 filter paper and the residue was washed several times with hot water until complete elimination of the alkalis. After washing, the residue was dried in an oven (Memmert, Germany) at 105 °C for 8 h and cooled in a desiccator and weighed (m1). The dry residue obtained was incinerated in a muffle furnace (Pyrolabo, France) at 550 °C for 3 h and cooled in a desiccator and then weighed again (m2). The calculation of diets fibers as a percentage of mass was performed using equation (4):

$$(\%)\ \text{Diets fibers} = [(m1\text{-}m2)\ /V] \times 100 \quad (4)$$

2.7. Statistical analysis

An analysis of variance was performed with the XLSTAT software (Version 2016; Adinosoft Inc.) and differences between mean values were determined by Tukey's test ($P < 0.05$). Principal component analysis (PCA) was performed using XLSTAT in order to visualize relationships among variables represented by tchapalo compounds. Possible relationships between antioxidant activities and the presence of phenolic compounds were investigated through Pearson correlation analysis (XLSTAT software).

3. Results

3.1. Physicochemical characteristics of sorghum wort and beers (BPC and BWY)

The physicochemical characteristics of sorghum wort and beers are given in Table 1. For all physicochemical parameters, the statistical analysis showed a significant difference between samples. The sorghum wort was characterized by a high pH (3.8 ± 0.24), high saccharification rate (57.39 ± 7.19%) and low acidity and ethanol content 0.65 ± 0.2% and 0.38 ± 0.21% respectively compared to the two beers. The beer produced from pure culture (*S. cerevisiae* F12-7) BPC, showed highest values of density, total soluble solids, pH, and water-soluble carbohydrates than those obtained with wild yeasts. On the other hand, the beer produced by wild yeasts showed significant higher titratable acidity and saccharification rate. There was no significant statistical difference in alcohol content of the two beers.

3.2. Phytochemical composition in sorghum wort and beers

Table 2 shows the phytochemical composition of sorghum wort and beer from pure culture of *Saccharomyces cerevisiae* (BPC) and beer from wild yeasts (BWY). Generally, for all phenolic compounds that were analyzed, their concentrations were higher in sorghum wort compared to both beers. The concentrations of total phenolic compounds were 1254.69 ± 2.3, 273.53 ± 4.23 and 239.68 ± 11.92 μg/mL GAE respectively for sorghum wort, BPC and BWY. These concentrations were significantly different. However, there were no significant statistical differences in the level of the others phenolic compounds, i.e. (total tannins, total flavonoids and total anthocyanins) between BPC and BWY. For these beers, the contents were 3680 ± 2.2 and 3600 ± 2.5 μg/mL, 39.21 ± 0.45 and 38.72 ± 0.29 μg/mL QE, $_{7.87\pm0.12}$ and 7 ± 0.62 μg/mL respectively for total tannins, total flavonoids, total anthocyanins.

Table 1
Physicochemical characteristics of sorghum wort and beers (BPC and BWY).

	Sorghum wort	BPC	BWY
Density	1.05 ± 0.00^a	1.03 ± 0.00^b	1.00 ± 0.00^c
pH	3.80 ± 0.20^a	3.54 ± 0.01^b	3.47 ± 0.01^c
Titratable acidity (%)	0.65 ± 0.20^a	0.73 ± 0.01^b	0.90 ± 0.02^c
Total Soluble Solids (°Brix)	15.60 ± 1.40^a	7.06 ± 0.11^b	2.36 ± 0.05^c
water-soluble carbohydrates (μg/mL)	253.00 ± 8.83^a	200.43 ± 4.50^b	63.21 ± 7.04^c
Saccharification rate (%)	57.39 ± 7.19^a	9.47 ± 2.50^b	22.21 ± 4.50^c
Ethanol (% v/v)	0.38 ± 0.21^a	4.30 ± 0.70^b	4.45 ± 0.18^b

Values are expressed as means ± standard deviation for three independent measurements. Means values with same letter in a line for each parameter are not significantly different ($P > 0.05$).

Table 2
Phytochemical composition in sorghum wort and beers.

	Total Phenols (μg/mL GAE)	Total Tannins (μg/mL)	Total Flavonoids (μg/mL QE)	Total Anthocyanins (μg/mL)
Sorghum wort	1254.69 ± 2.31[a]	13,035±1[a]	106.99 ± 2.94[a]	664.12 ± 1.56[a]
BPC	273.53 ± 4.23[b]	3680±2[b]	39.21 ± 0.45[b]	7.87 ± 0.12[b]
BWY	239.68 ± 11.92[c]	3600±2[b]	38.72 ± 0.29[b]	7.00 ± 0.62[b]

Values are means ± standard deviation, n = 3. Means in the same column with different letters are significantly different according to Tukey's test ($p < 0.05$).

3.3. Antioxidant activities

The antioxidant activities have been assessed from two methods: DPPH and FRAP. Table 3 shows the antiradical activity (DPPH) values in the samples. Antiradical activity was highest in the sorghum wort and lowest less in the BWY. The antiradical activity values were 73.33 ± 1.15, 59.62 ± 2.92 and 16.22 ± 2.09% respectively in sorghum wort, BPC and BWY and were statistically different. By contrast, the FRAP assay indicated highest antioxidant activity in BPC compared to sorghum wort and BWY. The values in sorghum wort BPC and BWY were 96 ± 6.74, 119.99 ± 8.01 and 106.25 ± 4.4 respectively. Statistical analysis showed significant difference between the value for BPC and the sorghum wort and BWY samples, but no significant difference between sorghum wort and BWY samples.

3.4. Correlation between parameter

A correlation matrix, presented in Table 4, was created to explore the possible relationships between the different parameters studied. A positive correlation was found between the phenolic compounds (total phenols, total tannins, total flavonoids, total anthocyanins) and antioxidant activity determined by DPPH method. However, there was a negative correlation between these compounds and antioxidant activity by FRAP method. Total phenol and DPPH were shown to have the highest correlation ($r = 0.704$, $P < 0.05$). The correlation between total anthocyans and FRAP was significant most higher but negative ($r = -0.720$, $P < 0.05$).

3.5. Principal component analysis of sorghum wort and beers (BPC and BWY)

The six measured variables of sorghum wort and beers produced were reduced to two main components (F1 and F2) by the PCA. F1 and F2 explain 100% of total data variance, with F1 alone accounting for 85.75% of the observed variations. The variables which mainly contributed positively (F loadings > 0.8) to F1 were total phenols, total tannins, total flavonoids, total anthocyanins, and FRAP was the variable which contributed negatively to F1. No variable was strongly correlated to F2 (Table 5). As shown Fig. 1, along F1, the samples could be separated into two groups. The first group was composed of beers (BPC and BWY) which were separated from the second group which was the sorghum wort.

Table 3
Antioxidant activities of sorghum wort and beers.

	DPPH (% Antiradical activity)	FRAP (μg/mL Ascorbic acid)
Sorghum wort	73.33 ± 1.15[a]	96.00 ± 6.00[a]
BPC	59.62 ± 2.92[b]	119.99 ± 8.01[b]
BWY	16.22 ± 2.09[c]	106.25 ± 4.40[a]

Values are means ± standard deviation, n = 3. Means in the same column with different letters are significantly different according to Tukey's test ($p < 0.05$).

Table 4
Correlation between phytochemical composition of sorghum wort and beers and antioxidant activities.

	Total Phenols	Total Tannins	Total Flavonoïids	Total Anthocyanins
DPPH	0.704[a]	0.680[a]	0.689[a]	0.685[a]
FRAP	−0.702[a]	−0.703[a]	−0.717[a]	−0.720[a]

[a] The correlation is significant at the 0.05 level.

3.6. Diets fibers and antimalarial compounds content

The diets fibers and antimalarial compounds content are presented in Table 6. Just as the phenolic compounds, the diets fibers had higher levels in sorghum wort than in the beers (BPC and BWY). The diets fibers contents were 0.565 ± 0049, 0.245 ± 0.007 and 0.47 ± 0.014 (g/100 mL) respectively in sorghum wort, BPC and BWY. In addition, statistical analysis showed a significant difference between the levels in all samples. Unlike diets fibers, no antimalarial compounds were found in the samples.

4. Discussion

The use of sorghum (*Sorghum bicolor*) in African traditional medicine had probably contributed to the market success of traditional sorghum beer. This beverage has been perceived by consumers as possessing the therapeutic qualities. In this context, there is a growing demand for this beer. As the fermentation process influences the formation of bioactive compounds of this beverage, the differences observed between their levels in sorghum wort and beers in this study could be attributed to fermentation process. During fermentation, the level of physicochemical parameters and bioactive compounds are strongly influenced by the metabolism of the yeasts. Indeed, it has been reported that some microorganisms, in particular yeasts, can degrade phenolic compounds which then serve them as carbon substrates and thus promote their growth (Macheix et al., 2005). From the phytochemical view a comparison of the two beers allows in a sense to highlight the performance of the *Saccharomyces cerevisiae* strain used in the study. In according Macheix et al. (2005), the yeasts are able to use the phenolic compounds for their growth. Thus, compared to the traditional inoculum composed of several microorganisms, *S. cerevisiae* strain seemed to degrade as many phenolic compounds as the mixture of microorganisms contained in the traditional inoculum did. Species belonging to genera *Saccharomyces, Candida, Kluyveromyces, Kloeckera* and *Torulaspora* have been identified in traditional sorghum beer by several authors (Konlani et al., 1996; Dedeh et al., 1999; N'guessan et al., 2010). Also, during the course of the alcoholic fermentation, the death of non-*Saccharomyces* yeasts is usually attributed to the lower ethanol tolerance of these yeasts by comparison to *S. cerevisiae* (Jolly et al., 2006). Thus, the phenolic compounds content particularly total phenols of the traditional sorghum beer seemed to be influenced by the inoculum contrary to others phenolic compounds in this study. According to several authors, the phenolic compounds have a synergistic effect on antioxidant activity (Goulas et al., 2010; Xie et al., 2015; Rahmanian et al., 2015). Antioxidant activity of the sorghum wort seemed related to phenolic compounds. There are no studies sighted in literature on traditional sorghum beer antioxidant activities. However a

Table 5
Rotated principal component loadings resulting by principal component analysis for sweet wort and beers (BPC and BWY).

	F1	F2
Total Phenols	1.000	0.012
Total Tannins	1.000	−0.010
Total Flavonoids	1.000	−0.011
Total Anthocyanins	1.000	−0.016
DPPH	0.698	0.716
FRAP	−0.811	0.585

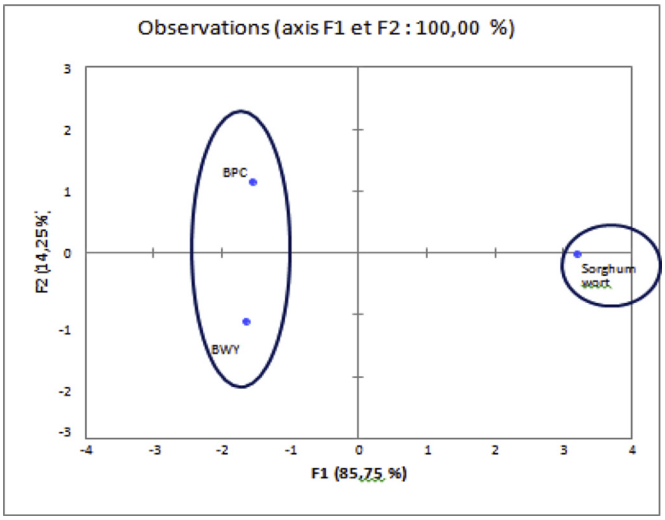

Fig. 1. Plot of the two principal components in PCA of sorghum wort and beers (BPC and BWY).

study on red sorghum grain antioxidant activity, the variety used for traditional sorghum beer preparation showed that the antioxidant activity was related to total phenols content (Boua et al., 2010). This same result has been observed in this study where the correlation between total phenols and antioxidant activity (DPPH method) was significant. For FRAP method, the results were expressed of ascorbic acid equivalents, thus the antioxidant activity value would depend to the ascorbic acid concentration in sample. Previous studies showed that ascorbic acid concentration was lower in the sorghum wort than sorghum beer. In fact, during alcoholic fermentation of traditional sorghum beer, the ascorbic acid content was increased (Aka et al., 2008a). This increasing would be due to the yeast metabolic activities, thus the microorganisms could influenced the antioxidant activity of traditional beverages fermented. *S. cerevisiae* strain used as starter in this study seemed to enhance the ascorbic acid production. Globally, compared to the several other studies the results of this study are interesting because of the higher antioxidant activity values. In addition, it is probable the therapeutic qualities of tchapalo would be due to the cereal used, which is sorghum. Indeed, according to Collin et al. (2000), sorghum was distinguished from other cereals used in breweries by its high content of phenolic compounds whose antioxidants properties are known. Furthermore, significant correlation between total phenols and antioxidant capacity (DPPH method) observed in this study had been demonstrated by many authors (Beretta et al., 2005; Ferreira et al., 2009; Bertoncelj et al., 2007). By contrast, the negative correlation observed between phenolic compounds and antioxidant capacity (FRAP method) observed in our study could revealed the importance of other phenolic groups in the antioxidant capacity measured by ferric reducing-antioxidant power (Fe^{3+}). The negative correlation observed in this study has been reported by some authors in their study on the antioxidant activity of medical plants (Bakchiche and Gherib, 2014; Albano and Miguel, 2011).

The diets fibers content is significantly different between each of the three beverages, with sorghum wort displaying the highest contents.

Table 6
Diet fibers and antimalarial compounds contents.

	Sorghum wort	BPC	BWY
Diets fibers (g/100 mL)	0.565 ± 0.049^{a}	0.245 ± 0.007^{b}	0.470 ± 0.014^{c}
Quinine formate	nd	nd	nd
Quinine dihydrochloride	nd	nd	nd
Chloroquine	nd	nd	nd

Values are means ± standard deviation, n = 3; Means on the same line with different letters are significantly different according to Tukey's test ($p < 0.05$). nd: not detected

This difference could mean that the microorganisms carrying out the fermentation could influence the level diets fibers as a result of the fermentation process. Also, the diets fibers concentration could decrease during the different step of beer production. However, comparatively to previous studies the values in this study were low. Enou (1997) and Kouame et al. (2015), have reported that the traditional sorghum beer could enhance the digestion. With the low content of diets fibers in the samples used in this study, the digestive property of beers could come from other compounds: phenolic compounds. Indeed, these compounds have been reported as compounds which enhance digestion (Gary, 2013; Tarko et al., 2013). However, all this remains to verify. Indeed, although the beers samples of this study contained the diet fibers and phenolic compounds but only the digestibility test could confirmed this property.

None of the antimalarial compounds investigated in this study (quinine formate, quinine dihydrochloride, chloroquine) could be found in the samples. Although some authors state that traditional sorghum beer has malaria curative properties according popular beliefs (Enou, 1997; Kouame et al., 2015), the results of this study do not support that. However, the high phenolic compounds contents and antioxidant activity found in tchapalo might be of interest as dietary supplements, and possibly in the prevention of several diseases. Also, the curatives properties attributed to traditional sorghum beer could be due to immuno-stimulation. These aspects will need to be further explored as part of future studies.

5. Conclusion

The traditional sorghum beer showed good antioxidant activities and the high phenolic compounds content. These parameters could be influenced by yeasts during fermentation. None antimalarial compounds have been found and the diets fibers concentrations were relatively lowest. Undoubtedly the therapeutic qualities attributed to traditional sorghum beer were due to phenolic compounds. Also, a study *in vivo* with animal model will most appropriate.

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Declaration of Competing Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Wahauwouélé Hermann Coulibaly: Writing - review & editing. **Koffi Maïzan Jean-Paul Bouatenin:** Writing - review & editing. **Zamblé Bi Irié Abel Boli:** Writing - review & editing. **Kouamé Kohi Alfred:** Writing - review & editing. **Youan Charles Tra Bi:** Writing - review & editing. **Koky Marc Celaire N'sa:** Writing - review & editing. **Marlène Cot:** Formal analysis. **Koffi Marcellin Djè:** Formal analysis.

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References

Aka, S., Camara, F., Zinzendorf, N.Y., Loukou, Y.G., Djè, K.M., 2008. Evaluation of organic acids and sugars contents during the production of "Tchapalo", a traditional sorghum beer in Côte d'Ivoire. J. Food Technol. 6, 189–195.

Aka, S., Djeni, N.T., N'guessan, K.F., Yao, K.C., Dje, K.M., 2008a. Variabilité des propriétés physico-chimiques et dénombrement de la flore fermentaire du "tchapalo", une bière traditionnelle de sorgho en Côte d'Ivoire. Afr. Sci. 4, 274–286.

Albano, S.M., Miguel, M.G., 2011. Biological activities of extracts of plants grown in Portugal. Ind. Crop. Prod. 33, 338–343. https://doi.org/10.1016/j.indcrop.2010.11.012.

Alves, C.Q., David, J.M., David, J.P., Bahia, M.V., Aguiar, R.M., 2010. Métodos para determinação de atividade antioxidante in vitro em substratos orgânicos. Quím. Nova 33, 2202–2210. https://doi.org/10.1590/S0100-40422010001000033.

Bakchiche, B., Gherib, A., 2014. Antioxidant activities of polyphenol extracts from medicinal plants in Algerian traditional pharmacopoeia. Int. J. Innovat. Appl. Stud. 9, 167–172.

Balole, T.V., Legwaila, G.M., 2009. *Sorghum bicolor* Moench. In: Belay, Brink M. (Ed.), PROTA (Plant Ressources of Tropical Africa). Wageningen. http://database.prota.org/search.htm.

Beretta, G., Granata, P., Ferrero, M., Orioli, M., Facino, R.M., 2005. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal. Chim. Acta 533, 185–191. https://doi.org/10.1016/j. aca.2004.11.010.

Bernfeld, P., 1955. Amylase α and β. In: Colowick N.O.K, S.P. (Ed.), Methods in Enzymology 1. Academic press, Inc, New York, pp. 149–158.

Bertoncelj, J., Doberšek, U., Jamnik, M., Golob, T., 2007. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem. 105, 822–828. https://doi.org/10.1016/j. foodchem.2007.01.060.

Boua, P.R., Abdoul-Latif, F., Kone, H.O., Bassole, N.I.H., Dicko, H.M., 2010, IXè edition. Étude des composés phénoliques et de l'activité anioxydante de 10 variétés de sorgho en Afrique de l'Ouest. Actes FRSIT, les communications, pp. 21–31.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Collin, S., Liégeois, C., Lermusieau, G., 2000. Measuring antioxydant efficiency of wort, malt and hops against the 2,2'-Azobis (2-amidinopropane) dihydrochloride-induced oxidation of an aqueous dispersion of linoleic acid. J. Agric. Food Chem. 48, 1129–1134. https://doi.org/10.1021/jf9911242.

Coulibaly, W.H., N'guessan, K.F., Coulibaly, I., Cot, M., Rigou, P., Djè, K.M., 2016. Influence of freeze-dried yeast starter cultures on volatile compounds of tchapalo, a traditional sorghum from Côte d'Ivoire. Beverages 2, 34–43.

Dedeh, Sefa, Sanni, S., Tetteh, A.I., Sakyi-Dawson, G., Yeasts, E., 1999. In the traditional brewing of 'pito' in Ghana. World J. Microbiol. Biotechnol. 15, 593–597. https://doi.org/10.1023/A:1008955300156.

Devi, S.P., Saravanakumar, M., Mohandas, S., 2011. Identification of 3-deoxyanthocyanins from red sorghum (Sorghum bicolor) bran and its biological properties. Afr. J. Pure Appl. Chem. 5, 181–193. http://www.academicjournals.org/AJPAC.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebes, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Enou, A.A.W., 1997. La bière de sorgho ou tchapalo : analyses, fabrication et consommation, approche analytique. Thèse de pharmacie de la faculté de pharmacie, Université de Cocody, Abidjan, p. 110.

Ferreira, I.C.F.R., Aires, E., Barreira, J.C.M., Estevinho, L.M., 2009. Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. Food Chem. 114, 1438–1443. https://doi.org/10.1016/j.foodchem.2008.11.028.

Gary, W., 2013. Possible effects of dietary polyphenols on sugar absorption and digestion. Mol. Nutr. Food Res. 57, 48–57. https://doi.org/10.1002/mnfr.201200511.

Goulas, V., Papoti, V.T., Exarchou, V., Tsimidou, M.Z., Gerthanassiss, L.P., 2010. Contribution of flavonoids to the overall radical scavenging activity of olive (*Olea europae* L.) leaf polar extracts. J. Agric. Food Chem. 58, 3303–3308. https://doi.org/10.1021/jf903823x.

Haggblade, S., Holzapfel, W.H., 1989. Industrialization of Africa's indigenous beer brewing. In: Steinkraus, K.H. (Ed.), Industrialization of Indigenous Fermented Foods. Marcel Dekker, (1989), New York, pp. 191–283.

Jolly, N.P., Augustyn, O.P.H., Pretorius, I.S., 2006. The role and use of non-*Saccharomyces* yeasts in wine production. S. Afr. J. Enol. Vitic. 27, 15–39.

Konlani, S., Delgenes, J.P., Moletta, R., Traoré, A., Doh, A., 1996. Isolation and physiological characterization of yeasts involved in sorghum beer production. Food Biotechnol. 1996 (10), 29–40. https://doi.org/10.1080/08905439609549899.

Kouame, K.B., Koko, A.C., Masse, D., Assidjo, N.E., 2015. Batch fermentation process of sorghum wort modeling by artificial neural network. Eur. Sci. J. 11, 75–93.

Lim, T.K., 2013. Edible Medicinal and Non-medicinal Plants, vol. 5. Springer science et business media, pp. 379–380.

Liuk, L., Sun, Y., Laura, T., Liang, X., Ye, H., Zeng, X., 2009. Determination of polyphenolic content and antioxydant activity of Kudingcha made from Ilex kudingcha C.J. Tseng. Food chem. 112, 35–41. https://doi.org/10.1016/j.foodchem.2008.05.038.

Macheix, J.J., Fleuriet, A., Jay-Allemand, C., 2005. Les composés phénoliques des végétaux : un exemple de métabolites secondaires d'importance économique. Presses polytechniques et universitaires romandes, p. 185.

Maoura, N., Mbaiguinam, M., Nguyen, H.V., Gaillardin, C., Pourquie, J., 2005. Identification and typing of yeast strains isolated from bili bili, traditional sorghum beer of Chad. Afr. J. Biotechnol. 4, 646–656.

Maoura, N., Mbaiguinam, M., Nguyen, H.V., Gaillardin, C., Pourquie, J., 2006. Suivi technique, analytique et microbiologique du bili bili, bière traditionnelle tchadienne. Afrique Sci. 2, 69–82.

Meda, A., Lamien, C.E., Romito, M., Millogo, J., Nacoulma, O.G., 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91, 571–577. https://doi.org/10.1016/j. food chem.2004.10.006.

N'guessan, F.K., N'Dri, D.Y., Camara, F., Djè, K.M., 2010. *Saccharomyces cerevisiae* and *Candida tropicalis* as starter cultures for the alcoholic fermentation of tchapalo, a traditional sorghum beer. World J. Microbiol. Biotechnol. 26, 693–699. https://doi.org/10.1007/s11274-009-0224-y.

N'guessan, K.F., Brou, K., Noemie, J., Casaregola, S., Djè, K.M., 2011. Identification of yeasts during alcoholic fermentation of tchapalo, a traditional sorghum beer from Côte d'Ivoire. Antonie Leeuwenhoek 99, 855–864. https://doi.org/10.1007/s10482-011-9560-7.

Rahmanian, N., Jafari, S.M., Wani, T.A., 2015. Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves. Trends Food Sci. Technol. 42, 150–172. https://doi.org/10.1016/j.tifs.2014.12.009.

Ribereau-Gayon, P., Stonestreet, E., 1965. Le dosage des anthocyanes dans le vin rouge. Bull. Soc. Chimie 9, 2649–2652.

Ribereau-Gayon, P., Stonestreet, E., 1966. Dosage des tanins du vin rouge et detérmination de leur structure. Chimie Anal 48, 188–196.

Sawadogo-Lingani, H., Lei, V., Diawara, B., Nielsen, D.S., Møller, P.L., Traoré, A.S., Jakobsen, M., 2007. The biodiversity of predominant lactic acid bacteria in dolo and pito wort, for production of sorghum beer. J. Appl. Microbiol. 103, 765–777.

Singleton, V., Orthofer, R., Lamuela-Raventoś, R., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Packer, L. (Ed.), Oxidants and Antioxidants, Part A, *Methods in Enzymology*, vol. 299, pp. 152–178.

Tarko, T., Duda-Chodak, A., Zając, N., 2013. Digestion and absorption of phenolic compounds assessed by in vitro simulation methods. Rocz. Panstw. Zakl. Hig. 64, 79–84.

van der Aa Kühle, A., Jespersen, L., Glover, R.L.K., Diawara, B., Jakobsen, M., 2001. Identification and characterization of *Saccharomyces cerevisiae* strains isolated from West African sorghum beer. Yeast 18, 1069–1079.

Wolf, Manuel d'analyses des corps gras, 1968. Azoulay Ed., Paris, France, p. 519.

Xie, P., Huang, L., Zhang, C., Zhang, Y., 2015. Phenolic compositions and antioxidant performance of olive leaf and fruit (*Olea europae* L.) extracts and their structure-activity relationships. J. Funct. Foods 16, 460–471. https://doi.org/10.1016/j.jff.2015.05.005.

Yao, A.K., Kadio, G., Coulibaly, A., Agbo, G.Z., 1995. Production du "Tchapalo'' a` partir du sorgho en Côte d'Ivoire. In: Processing and Industrial Utilization of Sorghum and Related Cereals in Africa. Proceeding of the OUA STRC-SAFGRAD Regional Symposium Ed. Microbiology, vol. 104, pp. 873–882.