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Research Article

Production and Biochemical, Phytochemical, Sensory, and Microbiological Characterization of "Bili-Bili" and "Cochette," Two Traditional Beers Produced from Sorghum (*Sorghum bicolor*) and Rice (*Oryza sativa*)

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"Bili-bili" and "cochette" are two traditional beers made from sorghum and rice, respectively. Despite the socioeconomical interest of these drinks, limited studies have been conducted to elucidate their quality and safety. To fill these gaps, the quality features and the safety status of both beers were assessed. After a reasoned field survey, the samples from various locations in Maroua were collected and analysed using referenced methods. The field survey revealed that both beers were produced under unsanitary conditions using rudimentary procedures. Mean values of pH (2.99 and 3.58), TTA (1.1 and 0.9%), alcohol (3.8 and 2.5%), DM (6.4 and 11.1%), TSS (6.94 and 6.18 °Brix), proteins (0.54 and 0.71 g/100 mL), amino acids (0.30 and 0.38 g/100 mL), and ash (1.52 and 0.51%) were recorded in "bili-bili" and "cochette," respectively. Similarly, the TPC, TFC, and carotenoid content of 325.5 and 352.4 mgGAE/100 mL, 314.4 and 278.9 mgQE/100 mL, and 95.4 and 89.4 mg/100 mL were noted in both beers, respectively. "Bili-bili" and "cochette" exhibited free radical scavenging activity of 42.4 and 36.7% and reducing power of 87.3 and 119.5 mgTE/100 mL, respectively. Overall acceptability ratings ranged from 5.6 to 7.5 and from 6.7 to 6.9 for "bili-bili" and "cochette," respectively. No pathogen was detected, but the presence of total aerobic bacteria, fungi, coliforms, and aerobic spore-forming bacteria flora above the recommended limits made both beers unsafe and potentially harmful for consumers. Given all above, improving of the production scheme and microbiological quality of the two traditional beers are required to ensure the safety of consumers.

1. Introduction

Fermentation is one of the oldest and most widely used methods for processing and preserving agricultural products, extending shelf life, improving taste, and increasing both functional and nutritional properties of fermented foods and beverages [1]. Fermented foods and beverages are now defined as food products made by controlled microbial development through enzyme conversion of food components. Traditional fermented foods and beverages have a vital role in many communities' cultures and customs, as well as in human nutrition. This group accounts for roughly one-third of human food and represents 20%–40% of the world food supply [2].

Beer is the oldest alcoholic fermented beverage and the third most consumed drink in the world, after water and tea [3]. Due to the scarcity of barley in many African countries, traditional beers are typically made from sorghum, millet, maize, or rice, with sorghum serving as the predominant cereal [4]. Sorghum is a staple food for more than 500 million people living in dry and semiarid areas [5] and provides essential nutrients and nutraceuticals such as phenolics and dietary fibers [6]. Sorghum beers are by far the most prized traditional cereal-based beers in Africa. They are

referred to as "dolo" in Burkina Faso and Mali [7], "tchapalo" in Côte d'Ivoire [8], "tchoukoutou" in Benin [9], and "pito" and "burukutu" in Ghana and Nigeria [10]. In northern Cameroon, fermented sorghum beers are known as "bili-bili." In the same region, fermented cereal beer is referred to as "cochette" when sorghum is partly or fully replaced with rice.

"Bili-bili" is the most vended traditional beer in Maroua. This opaque alcoholic drink is brewed both by young and adult women using a set of operations orally transmitted by ascendants to transform sorghum grain to beer. "Bili-bili" can be sweet, acidic, highly bitter, or bitter depending on the fermenting time. The latter is the more prevalent version of "bili-bili." "Cochette" is a traditional local beer brewed by combining unmalted cooked-rice flour with malted rice or malted sorghum. This fermented rice beer is mostly produced in Cameroon by Chadian women. "Cochette" is a low-alcohol rice beer since spontaneous fermentation happens without the addition of yeasts, as opposed to other high-alcohol yeast-fermented rice beers around the world such as "jou" and "zutho" from India, "sake" from Japan, "laochao" from China, "takju" from Korea, and "khaomak" from Thailand [11]. Both traditional beers, like many African beers, have a remarkable socioeconomic character because they are a significant source of income for brewers, grain producers, and sellers. In addition, they are widely consumed by underpaid, unemployed, or rural people due to their low cost [12].

The availability of low-cost foods and drinks produced with local knowledge and resources may aid in increasing dietary diversity, particularly among low-income households. As a result, recording of scientific information on the quality and safety of locally produced goods is advantageous. Several research studies in Cameroon have been undertaken in this area to describe traditional processing and to characterise the quality attributes of various types of fermented beverages [13–15]. However, there is no current scientific record on both traditional beers. There is little information regarding their quality features, and no data on the bioactive properties and microbial communities associated with both indigenous cereal beers are available. Therefore, the aim of this study was to document the traditional preparation and to determine the physicochemical profile, proximate composition, bioactive compounds, and antioxidant properties, as well as microbial quality and safety of "bili-bili" and "cochette" collected in various locations throughout Maroua, the headquarter of the far north Cameroon. The findings generated in this study can be used to guide consumer buying choices concerning traditional beers.

2. Materials and Methods

2.1. Reagents and Chemicals. All reagents and chemicals used in this study were of analytical grade. Folin–Ciocalteu reagent, gallic acid ($C_7H_6O_5$), quercetin ($C_{15}H_{10}O_7$), catechin ($C_{15}H_{14}O_6$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), aluminum chloride ($AlCl_3$), sodium bicarbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), and 3,5-dinitrosalicylic acid (DNSA) were gifted by the Integrated Center for Research, Expertise and Technological Transfer in Food Industry, Bioaliment TehnIA (Dunarea de Jos University of Galati, Romania). Ferric chloride ($FeCl_3$), 2,4,6-tris(pyridyl)-s-triazine (TPTZ), methanol (CH_3OH), and n-hexane (C_6H_{12}) were purchased from Fisher (New Jersey, USA). Bovine serum albumin, vanillic acid ($C_8H_8O_4$), ascorbic acid ($C_6H_8O_6$), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (Mumbai, India). Galactose ($C_6H_{12}O_6$), casein ($C_8H_{125}N_{22}O_{39}P$), ninhydrin ($C_9H_6O_4$), and alanine ($C_3H_7NO_2$) were obtained from Columbia Biosciences (UK). The culture media plate consisted of plate count agar (PCA), potatoes dextrose agar (PDA), eosine methylene blue agar (EMB), and mannitol salt agar (MSA) which were obtained from Liofilchem (Teramo, Italy). In addition, *Salmonella Shigella* agar (SS), slanetz and bartley agar (SlaBa), and trypticase sulphite neomycin agar (TSN) were obtained from Criterion (Hardy diagnostic, California, USA), Oxoid (Hampshire, UK), and Condalab (Madrid, Spain), respectively.

2.2. Presentation of the Study Zone. The study was conducted in Maroua, the headquarter of the Far-North region of Cameroon. The city of Maroua is divided into three districts, with a cumulated population of nearly 300,000 people working mostly in agriculture, cattle, and trade sectors. Commercial and craft center, Maroua is considered among the top ten cities of Cameroon. The climate is tropical, dry, and hot, with yearly average temperature and rainfall of 28.3°C and 794 mm, respectively. The area is mostly populated by the Guizga, Mofu, and Fulani living peacefully among other communities such as Mandara, Massa, Toupouri, Mousgoum, and Kotoko. The main food crops cultivated in the zone are cereals such as sorghum, millet, maize, and rice, as well as market gardening including onion, tomato, pepper, okro, and leafy vegetables. The zone was chosen for its cosmopolitan character, high frequency of production, and diversity of traditional cereal-based beers.

2.3. Survey on the Production of "Bili-Bili" and "Cochette". The survey on the production of both traditional beers was conducted between February and March 2021 in five locations as shown in Figure 1. They included Pont-vert (10°35′47.579″N and 14°20′19.727″E), Domayo (10°35′26.628″N and 14°18′41.712″E), Pitoaré (10°35′40.080″N and 14°15′00.582″E), Palar (10°36′07.284″N and 14°17′23.759″E), and Ouro-tchédé (10°35′02.549″N and 14°16′51.498″E). The places were chosen because they were the key production zones for both beers. The survey was oriented using a structured questionnaire that addressed sociocultural aspects of production, raw material used, main production stages, conditions and factors influencing the production, and adverse effects after consumption of the beverages. The survey was carried out in two levels. Production sites and cabarets were investigated at the primary level, while individual and groups of respondents were interviewed with producers and consumers at the secondary level. The exact number of production sites and cabarets present in

Maroua was determined after a preliminary survey, while the number of people to be interviewed was estimated based on the total population of the study zone, from which we deducted 65% of Muslims and 42% of those under 16 who do not consume alcoholic drinks. To the remaining population, we applied a 95% confidence level and 5% margin of error to calculate the size of population to be interviewed. At the end of the day, 12 sites of production and 50 cabarets were visited and 22 producers and 120 consumers were interviewed.

2.4. Collection of Samples. The sampling was carried out between May and June 2021, during the dry season. It was oriented from the main producers and retailers, which were located at various areas of the study zone depending on the type of traditional beer. Each site was sampled on a daily basis, and each sample was tasted before collection to confirm its freshness. So, the samples, with a volume of 350 mL each, were collected fresh on the day of handling, placed into sterile plastic bottles, sealed, labelled, and stored in icebox containers midfilled with lump ice. Four batches of 10 "bili-bili" samples each were collected from Palar, Domayo, Ouro-tchédé, and Pont-vert. Furthermore, 16 "cochette" samples were collected from Pitoaré and 2 lots of 12 samples each from Palar 1 and Palar 2. The Palar area was divided into two subsites because "cochette" beer was produced with either malted rice (Palar 1) or malted sorghum (Palar 2). Finally, 40 "bili-bili" and 40 "cochette" samples were collected and transported aseptically under cold regime to the laboratory for physicochemical, phytochemical, sensory, and microbiological analyses.

2.5. Physicochemical Analyses. The pH value was directly measured with a calibrated portable pH meter (Eco Testr, Singapore) [16]. The total titratable acidity and volatile acidity were determined by using the titrating method as previously described by Kitessa et al. [17] and Basumatary et al. [18], respectively. Twenty-five millilitres of the beer sample were pipetted and poured into a beaker, 3 drops of 5% phenolphthalein were added, and the sample was titrated with 0.1 N sodium hydroxide solution (NaOH) until the persistence of pink color. The total titratable acidity value expressed in % of lactic acid and the volatile acidity reported as % of acetic acid were calculated using the following equations:

$$\%\ \text{Lactic acid}\left(\frac{\text{wt}}{v}\right) = \frac{\left[V_{\text{NaOH}}\,(\text{mL}) \times N_{(\text{NaOH})}\,(0.1) \times 90.08\right]}{[V\ \text{sample}\,(\text{mL}) \times 10]}, \tag{1}$$

$$\%\ \text{Acetic acid}\left(\frac{v}{\text{wt}}\right) = \frac{\left[V_{\text{NaOH}}\,(\text{mL}) \times N_{(\text{NaOH})}\,(0.1) \times 6\right]}{\text{Weight of sample}\,(\text{g})}. \tag{2}$$

The electrical conductivity (EC), total dissolved solids (TDSs), and temperature were recorded immediately after sampling by using a digital multifunctional portable conductivity meter (e-1 TDS and EC, Shenzen, China). The mean values of triplicate measurements were expressed in μS/cm, ppm, and °C, respectively. The total soluble solids (TSSs) were measured with a portable ATC refractometer (RHB 90, Shenzhen, China) that was regularly calibrated with distilled water. The recorded values were expressed in °Brix. The specific gravity was determined using the AOAC [19] method by dividing the ratio weight/volume of the beer sample by the same ratio for distilled water according to the following formula:

$$\text{Specific gravity}\,(SG) = \frac{(\text{Weight of sample/Volume of sample})}{(\text{Weight of water/Volume of water})}. \tag{3}$$

The alcohol content of the beer samples was estimated with the calculation method using the following formula that incorporates both TSS and SG measurements as previously reported by Bayoï and Etoa [20]:

$$\text{Alcohol content}\left(\%, \frac{v}{v}\right) = \text{TSS-}\,[(\text{SG} - 1) \times 100]. \tag{4}$$

The dry matter content was determined by drying the samples in a forced-air oven (Memmert, Germany) at 105°C to a constant weight [12]. The dry matter value, expressed in percent mass by mass (%, m/m), was calculated using the following formula:

$$\text{Dry matter content}\left(\%, \frac{m}{m}\right) = \left[\frac{\left(\text{Weight}_{\text{dried sample+beaker}}\,(g) - \text{Weight}_{\text{empty beaker}}\,(g)\right)}{\left(\text{Weight}_{\text{no dried sample+beaker}}\,(g) - \text{Weight}_{\text{empty beaker}}\,(g)\right)}\right] \times 100. \tag{5}$$

2.6. Proximate Analysis of Traditional Beers. The soluble protein content of "bili-bili" and "cochette" was determined using the Bradford colorimetric method based on the principle of protein-dye binding [21]. The free amino acid content was quantified by ninhydrin reaction with alanine as reference [22]. The soluble protein content was expressed in g of BSA equivalent per 100 mL (g BSAE/100 mL) using the BSA standard curve ($R^2 = 0.9809$). The free amino acid

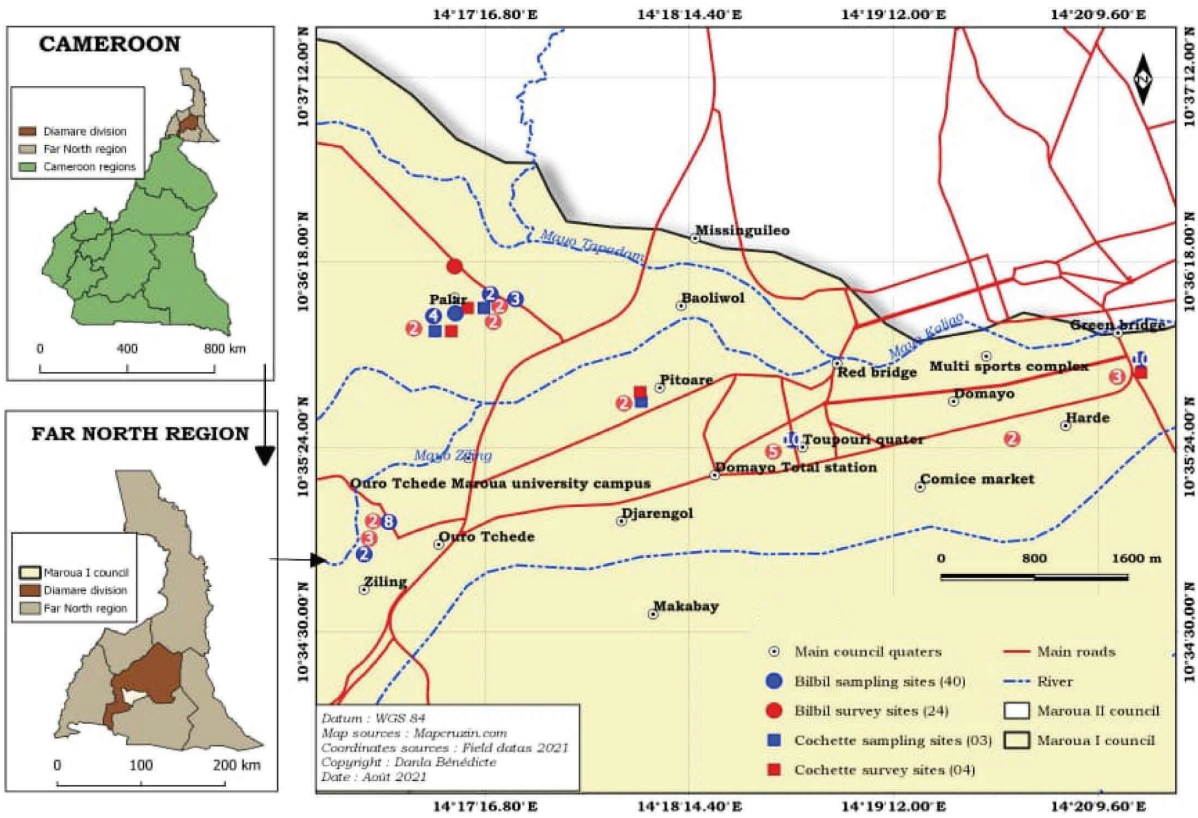


Figure 1: Mapping of the survey and sampling sites.

content was expressed in g of alanine equivalent per 100 mL of beer (g AE/100 mL) from the alanine calibration curve ($R^2 = 0.9951$).

Total sugars of the samples were determined using the phenol-sulfuric method as described by Nielsen [23], while reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNSA) method as reported by Jain et al. [24] with slight modifications. The glucose standard curve ($R^2 = 0.9806$) was used to determine the total sugar content expressed in g of glucose equivalent per 100 mL of beer (g GlcE/100 mL), while the galactose calibration curve ($R^2 = 0.9972$) was considered to quantify the reducing sugar content expressed in g of galactose equivalent per 100 mL of beer (g GalE/100 mL).

The ash content was evaluated using Pigozzi et al.'s [25] method with minor modifications by incinerating the samples in a muffle furnace (Nabertherm, Germany) at 550°C to reach the constant weight. The ash value was calculated in percentage mass by mass (%, m/m).

2.7. Measurement of Bioactive Compounds

2.7.1. α-Carotene, β-Carotene, Lycopene, and Lutein Contents. The content of each carotenoid was determined as described by Sumanta et al. [26] with slight modifications. Each beer sample (0.1 mL) was placed in a glass tube and 2.5 mL of hexane containing 1% of ascorbic acid (5%) was added. After vortexing, 1.5 mL of acetone was added and the mixture was still homogenized for 15 minutes. Subsequently, 2.5 mL of methanol was added and the reacting tube was incubated in darkness at room temperature for 3 h. Then, the organic phase was collected and the aqueous phase was extracted once again with 2.5 mL of hexane. At the end of the day, both organic phases were combined and filtered through Whatman filter paper no. 4, and the absorbances were read at 445, 446, 450, and 472 nm with a UV-Vis spectrophotometer (Jenway 7305, Bibby Scientific, Staffordshire, UK). α-carotene, β-carotene, lutein, and lycopene contents were calculated according to the following formula:

$$\alpha\text{–carotene}, \beta\text{–carotene}, \text{lutein, and lycopene}\left(\frac{\text{mg}}{100\text{mL}}\right) = \frac{(\text{OD} \times V \times d)}{\left(\text{Ws} \times E^{1\%}\right)}, \quad (6)$$

where OD is the optical density; V is the total volume of the extract (mL); d is the dilution factor; Ws is the weight of the sample (mg); and $E^{1\%}$ is the absorption coefficient of α-carotene (2725 cm^{-1}), β-carotene (2592 cm^{-1}), lutein (2550 cm^{-1}), and lycopene (3450 cm^{-1}).

2.7.2. Total Phenolic Content (TPC). The total phenolic content of the samples was determined using the Folin–Ciocalteu method as previously described by Fu et al. [27] with minor modifications. The diluted sample (0.2 mL) was added to 1 mL of Folin–Ciocalteu reagent and 1 mL of 20% sodium carbonate. The mixtures were incubated at room temperature in darkness for 30 min before being measured for absorbance against blank at 765 nm using a UV-Vis spectrophotometer. A calibration curve ($y = 0.0031x + 0.0013$, $R^2 = 0.9883$) was established using gallic acid at various concentrations (0–250 μg/mL), and the TPC was expressed as mg of gallic acid equivalent per 100 mL of beer (mg GAE/100 mL).

2.7.3. Total Flavonoid Content (TFC). The total flavonoid content (TFC) of the samples was determined using the aluminium chloride method as described by Lakenbrink et al. [28] with slight modifications. Precisely, 1 mL of the sample was mixed with 0.2 mL of distilled water and 0.5 mL of 10% aluminium chloride. After 1 min, 2 drops of 1% acetic acid were added to the mixture and the absorbance against blank was determined at 430 nm with a UV-Vis spectrophotometer. Quercetin solutions at 0 to 250 μg/mL were used to establish the standard curve ($y = 0.0022x - 0.0117$ and $R^2 = 0.9964$), and the results were expressed as mg of quercetin equivalent per 100 mL of beer (mg QE/100 mL).

2.7.4. Total Condensed Tannin Content (TTC). The content of condensed tannin (TTC) of the samples was measured using the vanillin method as described by Shewakena et al. [29]. Each sample (0.5 mL) was added to 1.5 mL of acidified vanillin solution (1 g of vanillin in 100 mL of concentrated HCl). After 2 min of homogenising, the mixture was kept in the dark at room temperature for 5 min to develop the red-colored complex. The absorbance against blank was measured at 500 nm with a UV-Vis spectrophotometer. The TTC was reported as mg of catechin equivalent per 100 mL of beer (mg CE/100 mL) from a catechin standard curve ($y = 0.0027x$ and $R^2 = 0.9871$).

2.8. Antioxidant Properties of "Bili-Bili" and "Cochette"

2.8.1. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Assay. The radical scavenging activity of "bili-bili" and "cochette" samples was determined using the DPPH assay according to the modified protocol described by Floegel et al. [30]. Each sample 10-fold diluted (0.5 mL) was added to 1.5 mL of the DPPH reagent in absolute ethanol in the ratio 1 : 10, and the mixture was left in the dark at room temperature for 15 min. The absorbance was read at 517 nm, and the DPPH scavenging activity expressed in percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging activity } (\%) = \left[1 - \left(\frac{\text{Abs of sample}}{\text{Abs of DPPH}}\right)\right] \times 100. \quad (7)$$

Abs of the sample is the absorbance of the beer sample mixed with the DPPH solution, and Abs of the DPPH is the absorbance of DPPH solution at 517 nm.

The absorbance values were also used to determine the total antioxidant capacity expressed as mg of Trolox equivalent per 100 mL of beer (mg TE/100 mL) using a calibration curve ($y = 0.0043x + 0.0259$ and $R^2 = 0.9617$) of Trolox at various concentrations (0–120 μg/mL).

2.8.2. Ferric Reducing Antioxidant Power (FRAP) Assay. The ferric reducing antioxidant power was evaluated according to the method described by Thaipong et al. [31] with minor modifications. Prior to the analysis, the samples were 20-fold diluted and 0.5 mL of each sample was mixed with 1.5 mL of the FRAP reagent containing TPTZ (10 mM), acetate buffer (300 mM, pH 3.6), and $FeCl_3$, 6 H_2O (20 mM) in the ratio 10 : 1 : 1. After 5 min of incubation at room temperature in the dark, the absorbance against the blank was measured at 593 nm with a UV-Vis spectrophotometer. The results were expressed as mg of Trolox equivalent per 100 mL (mg TE/100 mL) using a standard curve ($y = 0.0075x - 0.0139$ and $R^2 = 0.9979$) of Trolox at the concentrations ranging from 0 to 120 μg/mL.

2.8.3. Phenolic Antioxidant Coefficient and the Relative Antioxidant Capacity Index. Both parameters were used to achieve more comprehensive comparison of the antioxidant activity among the various samples analysed.

(1) Phenolic Antioxidant Coefficient (PAC). The phenolic antioxidant coefficient (PAC) of each beer sample was determined using the results of DPPH, FRAP, and TPC assays as previously described by Petrović et al. [32]. The PAC was calculated as the ratio between the particular antioxidant activity (AOA) and TPC according to the following formulas:

$$\text{PAC}_{\text{DPPH}} = \frac{\text{AOA from DPPH assay}}{\text{TPC}}, \quad (8)$$

$$\text{PAC}_{\text{FRAP}} = \frac{\text{AOA from FRAP assay}}{\text{TPC}}. \quad (9)$$

(2) Relative Antioxidant Capacity Index (RACI). The relative antioxidant capacity index (RACI) is a comprehensive method for ranking the antioxidant activity of different food samples. The RACI was determined by giving equal weight to both antioxidant assays, including the TPC considered as the reducing assay as described by Sun and Tanumihardjo [33]. This unitless index was calculated in two steps. To begin, the antioxidant activity values in each dataset are transformed into dimensionless values known as standard scores calculated using the following formula:

$$\text{Standard score} = \frac{(x - \mu)}{\sigma}, \tag{10}$$

where x is the raw data, μ is the mean value, and σ is the standard deviation.

Then, the RACI was calculated by averaging the standard scores of a given sample.

2.9. Microbiological Analyses and Determination of the Safety Status of "Bili-Bili" and "Cochette". The microbial counts of "bili-bili" and "cochette" samples were performed in accordance with the microbiological guidelines. According to the standard ISO 6887-1: 2017 [34], the samples were serially diluted with sterile saline water (0.85% NaCl). Fifty millilitres of each sample were added separately to 450 mL of thiogluconate broth, and the suspensions were mixed for 10 minutes before incubating at 30°C for 16 hours. Following the revivification, serial 10-fold dilutions from 10^{-1} to 10^{-8} were aseptically done using 1 mL of the preincubated sample and 9 ml of sterile saline water (0.85% NaCl). Then, 0.1 mL of the appropriate dilution was spread plated in triplicate on the specific sterile agar culture media and the inverted inoculated plates were incubated under the conditions specified in Table 1.

The safety status of traditional beer samples was assessed using the microbial scores endorsed on the microbial counts recorded during the microbiological analysis as previously described by Cuq [44] and Bayoï and Etoa [45]. Using a microbiological quality scale, the microbial counts were compared to referenced intermediate values (3 m, 10 m, and 1000 m), all endorsed on the standard count (m). Then, a microbial score ranging from 0 to 46 was assigned to each microbial count. The safety score was computed by summing up all the microbial scores recorded from the various microbial counts that resulted from the examination of samples gathered in the same location. Based on the safety score, the beer was rated as excellent when it had a score of 0 and as unsafe when it had a score of 45 or above.

2.10. Sensory Analysis. The sensory evaluation of traditional beer samples from several Maroua locations was carried out using an acceptance hedonic test, as previously described by Bayoï et al. [46]. An experienced panel of ten male judges (aged 18–27 years) was recruited. Before being informed about the purpose of the study and the experimental protocols, each panellist was requested to sign a consent form. The testing started at 08 am, with suitable lighting (25 w lamps), humidity (60–65%), and room temperature (25–30°). A sufficient distance was maintained to preclude communication between the panellists. Each fasting panellist received 30 mL of traditional beer in a cup labelled with random three-digit code, as well as another cup containing mineral water (30 mL) used to rinse their mouth before moving to the next sample. The panellist rated the dislike or like degree of each sample by scoring six sensory attributes (bitterness, alcoholic taste, texture/viscosity, odor, color, and overall acceptability) using a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely).

2.11. Statistical Analysis. All measurements were carried out in triplicate and the results were presented as the mean ± standard deviation. Primary data collected using a structured questionnaire were analysed by descriptive statistics. To determine the difference between the various beer samples, the one-way analysis of variance (ANOVA) plus post hoc Tukey honest significant difference (HSD) were used and the differences were considered significant at $p < 0.05$. The correlations between the measured parameters of the beer samples were determined using the Pearson correlation coefficient (r), and the recorded coefficients were used to design heat map correlation matrices using the ChiPlot online database available at https://www.chiplot.online (accessed on 16 October 2023). Principal component analysis (PCA) was used to determine the relationship between the measured variables and the beer samples from various places.

3. Results and Discussion

3.1. Sociodemographic Characteristics of "Bili-Bili" and "Cochette" Producers. Table 2 summarises the results of surveys on "bili-bili" and "cochette" producers and some key information about the traditional processing of both beers. The findings revealed that all the producers of both traditional beers are female (100%). This was not surprising given that cooking is traditionally a woman matter in Africa. This observation was previously reported by Madilo et al. [47], Adinsi et al. [48], and Sacca et al. [49] that worked on the traditional production of "aliha" (a Ghanaian fermented maize-based beverage), "gowe" (an indigenous fermented cereal-based drink from Benin), and "akpan" (a West Africa traditional yoghurt-like cereal product), respectively. Most "Bili-bili" and "cochette" producers come from Cameroon (69.2%) and Chad (100%), respectively. The principal Cameroonian tribes involved in "bili-bili" production are identified as Tupuri (leading tribe), Giziga, Moundang, and Mofu, while Ngambaye and Sara are the leading Chadian ethnic groups concerned with "cochette" production. This indigenous beer is mainly produced by Chadian because it was imported from Chad, bordering to Cameroon in the northern side. Indeed, both countries share 1,116 km of border, with the famous Lake Chad as the natural boundary.

The majority of "cochette" and "bili-bili" producers in Maroua were young, with ages ranged from 20 to 30 years (83.3%) and 30 to 40 years (53.8%), respectively. The bulk of "bili-bili" producers had been doing it for 2–4 years (69.3%),

Table 1: Standards, culture media, incubation conditions, and colonies features used for microbial analysis of "bili-bili" and "cochette" samples collected in various localities of Maroua.

Microbial groups	Standards	Culture media	Temperature and incubation time	Colonies features	References
Total aerobic mesophilic bacteria	ISO 4833-2: 2013	Cycloheximide (2.5 mg/L) plate count agar (PCA)	35°C for 48 h	Growth	[35]
Total fungi	ISO 21527-1: 2008	Chloramphenicol (0.02%) potatoes dextrose agar (PDA)	25°C for 96 h	Creamy (yeasts); filamentous and colored (moulds)	[36]
Total coliforms	ISO 4832: 2006	Eosine methylene blue agar (EMB)	35°C for 24 h	Dark violet	[37]
Fecal coliforms	ISO 9308-1: 2000	Eosine methylene blue agar (EMB)	44°C for 24 h	Purplish red	[38]
Aerobic mesophilic spore-forming bacteria	SP-VG M 008-3 (3): 1998	PCA (after heating at 80°C for 10 min)	35°C for 48 h	Growth	[39]
Staphylococci spp.	ISO 6888-1: 2021	Mannitol salt agar (MSA)	37°C for 24 h	Yellow	[40]
Salmonella/Shigella	ISO 6579-1: 2017	Enrichment in selenite cysteine broth + SS agar	37°C for 24 h	Colorless, black-centred	[41]
Fecal *Streptococci*	ISO 7899-2: 2000	Cycloheximide (0.5%) Slanetz–Bartley agar (SlaBa)	37°C for 48 h	Red or Brown	[42]
Sulphite-reducing *Clostridia*	ISO 15213: 2003	Trypticase sulphite neomycin agar (TSN)	46°C for 48 h (anaerobiosis)	Small and dark	[43]

Table 2: Overall information from "bili-bili" and "cochette" surveys.

Parameters	"Bili-bili"	"Cochette"
Producers	*Percent*	*Percent*
Gender	Female (100%)	Female (100%)
Origin of producers	Cameroon (69.2%); Chad (30.8%)	Chad (100%)
Age of producers (years)	[20–30] (46.2%); [30–40] (53.8%)	[20–30] (83.3%); [30–40] (16.7%)
Experience (years)	2 (15.4%); 3 (30.8%); 4 (23.1%); 5 (7.7%); 6 (15.3%); 7 (0%); 8 (7.7%)	1 (50%); 2 (30.3%); 3 (19.7%)
Production sites	"Pont-vert" (28%); Domayo (27%); Palar (19%); Ouro-tchédé (14%); Pitaore (6%); Harde (6%)	"Pont-vert" (16.7%); Palar (50%); Pitoare (33.3%)
Potential cereals used	Sorghum (red, white, and yellow varieties); maize	Rice (variety from Yagoua); sorghum
Main cereals used	Yellow (80%) and red (20%) sorghum	Rice (100%)
Supply period	All the year (53.8%); February-March (7.7%); February-March-October-November (15.4%); February-March-November (15.4%); February-October-November (7.7%)	All the year (100%)
Seeds condition during processing	Malted (100%)	Not malted (83.3%), malted (16.7%)
Production critical stages	Malting-cooking (38.4%); malting-cooking-fermentation (15.4%); cooking (15.4%); cooking-fermentation (15.4%); malting-fermentation (7.7%); fermentation (7.7%)	Fermentation (66.6%); cooking fermentation (16.7%); none (16.7%)
Fermentation time (h)	9 (15.4%); [9-10] (53.8%); 10 (23.1%); 11 (7.7%)	8 (66.6%); [8-9] (16.7%); 9 (16.7%)
Production time (days)	6 (69.2%); 7 (23.1%); [6–8] (7.7%)	[4-5] (16.7%); 5 (93.3%)
Producers' incomes (%)	50 (69.2%); 50–75 (7.7%); 75–100 (7.7%); depending on the market (15.4%)	50 (83.3%); 50–100 (16.7%)
Consumers	*Percent*	*Percent*
Consumption rate	93.1%	6.9%
Factors that provide maturity of the product	Alcohol content and foam (30.8%); color, alcohol content, and bitterness (23.1%); bitterness and color (23.1%); bitterness (15.4%); color and the alcohol content (7.6%)	Alcohol content (66.6%); alcohol content and foam (16.7%); bitterness (16.7%)
Health adverse effects after consumption of the beverage	Vomiting (33.3%); diarrhoea (33.3%); headaches (33.3%)	Diarrhoea (50%); headaches (50%)

whereas all "cochette" producers had less than 3 years of expertise (100%). This suggests that the production of traditional beers in Maroua is a highly sustainable venture because all the processors were under 40 years old (young) and have less than 10 years of experience [47, 49]. According to the survey results, both indigenous beers represent a source of income for the producers. The majority of "bili-bili" (69.2%) and "cochette" (83.3%) producers said this activity provided 50% of their income.

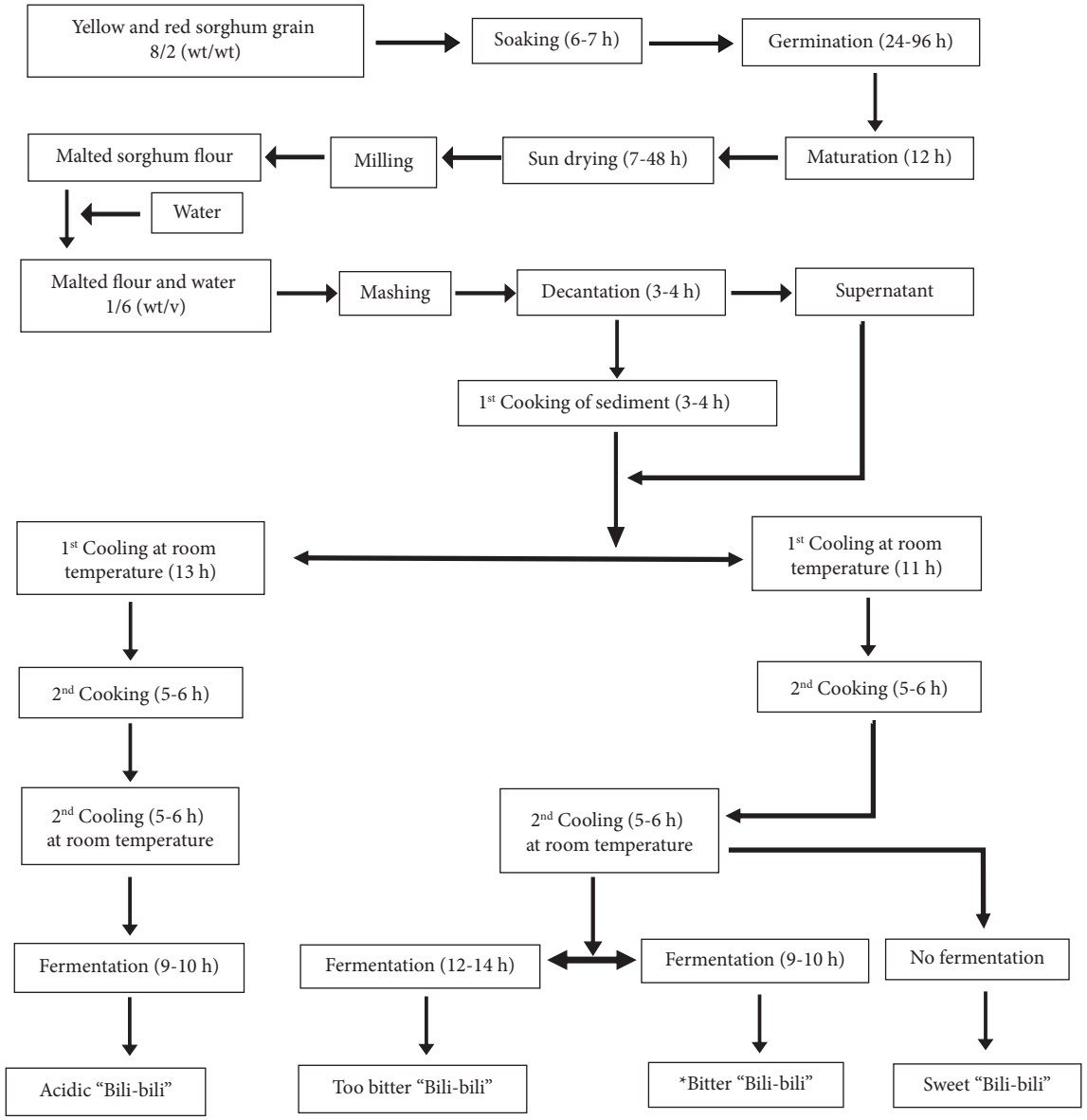
The two traditional beers are popular among unemployed and low-income people. However, "bili-bili" (93.1%) is ingested more frequently than "cochette" (6.9%). This is explained by the fact that "bili-bili" is considered as a local drink by Cameroonian despite the fact that it is commonly produced in Chad [50] while "cochette" is regarded as an imported traditional beer from Chad. The primary production sites of "bili-bili" were located at Pont-vert (28%), Domayo (27%), Palar (19%), and Ouro-tchédé (14%), whereas Palar (50%) and Pitoaré (33.3%) were identified as the main production points of "cochette."

The majority of "bili-bili" consumers (77%) cited the alcohol content, foaming ability, color, and bitterness as the sensory attributes that more appeal to "bili-bili" costumers, whereas most "cochette" consumers pointed out the alcohol content as the main sensory attribute of this traditional beer. Vomiting (33.3%), diarrhoea (33.3%), and headaches (33.3%) were reported as side effects after "bili-bili" ingestion. The most common health consequences associated with "cochette" ingestion were diarrhoea (50%) and headaches (50%).

3.2. Characterization of the Traditional Processing of "Bili-Bili" and "Cochette". Figure 2 depicts a consensus flowchart (A and B) and pictorial (C and D) of the traditional production of "bili-bili" and "cochette." As shown in Table 2, sorghum and rice were identified by producers as the primary cereals required for the processing of "bili-bili" and "cochette," respectively. When the "bili-bili" is processed with a single cereal, the producers primarily used either yellow sorghum (45%), red sorghum (30%), or white sorghum (20%). Maize (5%), considered as minor cereal, is not appreciated as the base ingredient by "bili-bili" producers because they reported that customers complain about headaches after the ingestion of maize-based beers. "Bili-bili" producers prefer to use the mixture of yellow (80%) and red (20%) sorghum because both varieties are available on the market all over the year (Table 2) and yields a beer appealed by the custumers. The use of sorghum mixture contradicts the findings of a survey conducted by Charles et al. [51] on the "bili-bili" processing. According to these authors, most "bili-bili" brewers in northern Cameroon employed red "djigari" sorghum as the base cereal. This could be explained by differences in the number of producers surveyed and the study zone. The survey of the current work was conducted in a limited sample of producers in Maroua, while the previous work was carried out in a large sample of producers from Maroua, Garoua, and Ngaoundéré, the main cities of the northern part of Cameroon.

During the survey, four variants of "bili-bili" were identified based on taste including the bitter "bili-bili" (considered for the current study) known as "mbalwelli" in Fufuldé, "ïmadédigui" in Tupuri, "mbasla" in Giziga, "nyimiyan" in Moundang, "zoum" in Mafa, and "mahai" in Mufu. Sara and Ngambaye, two Chadian ethnic groups, refer to it as "kass" and "kido," respectively; the very bitter "bili-bili" is called "yiglague" and "nyimizoké" in Tupuri and Moundang, respectively; the acidic "bili-bili" is referred to as "yibradé" in Tupuri, "nyimibonré" in Moundang, and "corréc" in Giziga; and the sweet "bili-bili" is also named "dakam" and "das" in Fulfuldé and Tupuri, respectively. These variations were mostly found during the cooling and fermentation stages (Figure 2(a)).

All "bili-bili" producers soak the grains for 6–7 h at room temperature, before allowing them to germinate for 1–3 days. The grains are watered twice a day, morning and night. According to the producers, the germination time varies between cereals. Red sorghum requires 2 days to germinate, yellow and red sorghum 3 days, and red sorghum and maize 3-4 days. In general, the germination time should be long enough to allow a large proportion of grains to germinate [52]. The germination time required during the processing of "bili-bili" was alike to that requisite for the production of "dolo" (sorghum-based beer from Benin), "tchakpalo" (cereal-based beer from Benin and Togo), "tchapalo" (sorghum-based beer from Côte d'Ivoire), "red kapsiki" (red sorghum-based beer from far north Cameroon), "pito" (Ghanaian and Nigerian cereal-based beer), and "dora-bonga" (sorghum-based beer from Central Africa Republic) [1]. The sprouted grains (as depicted in Figure 2(c) d) are sun-dried for 2-3 hours before 12 hours of maturation. The germinated kernels are sun-dried for periods ranging from 7 to 48 hours, depending on the climatic conditions such as sunshine intensity and wind speed. Sun drying (as illustrated in Figure 2(c) e) terminates the traditional malting stage and reduces the amylase activity in sprouted kernels [53]. As shown in Table 2, the malting process was identified as a critical stage in the production of "bili-bili" by the producers interviewed. Taylor and Dewar [54] stated that when the malting stage is not properly controlled, the malt is very poor and the beer quality suffers. The germinated and dried grains are milled into malted flour and mixed with water (6 L/kg of sorghum) before being allowed to settle for 3-4 hours. The supernatant is recovered after decantation, and the floury bottom is precooked for 3-4 hours until the red color appears. The previous supernatant is added to the precooked deposit, and the wort is allowed to cool at room temperature for 11 hours. The wort was cooked either for further 5-6 hours or until the foam has completely disappeared (Figure 2(c) g). In addition to malting, cooking was identified as another critical stage during "bili-bili" processing by many producers. This is consistent with the previous results reported by Bayoï and Etoa [55] who pointed out malting, cooking, and fermentation as the major critical stages during the production of "té" and "mpedli," two traditional sorghum beers from northern Cameroon. As shown in Table 2, most surveyed producers (38.4%) identified malting and cooking as critical stages during "bili-bili"



(a)

Figure 2: Continued.

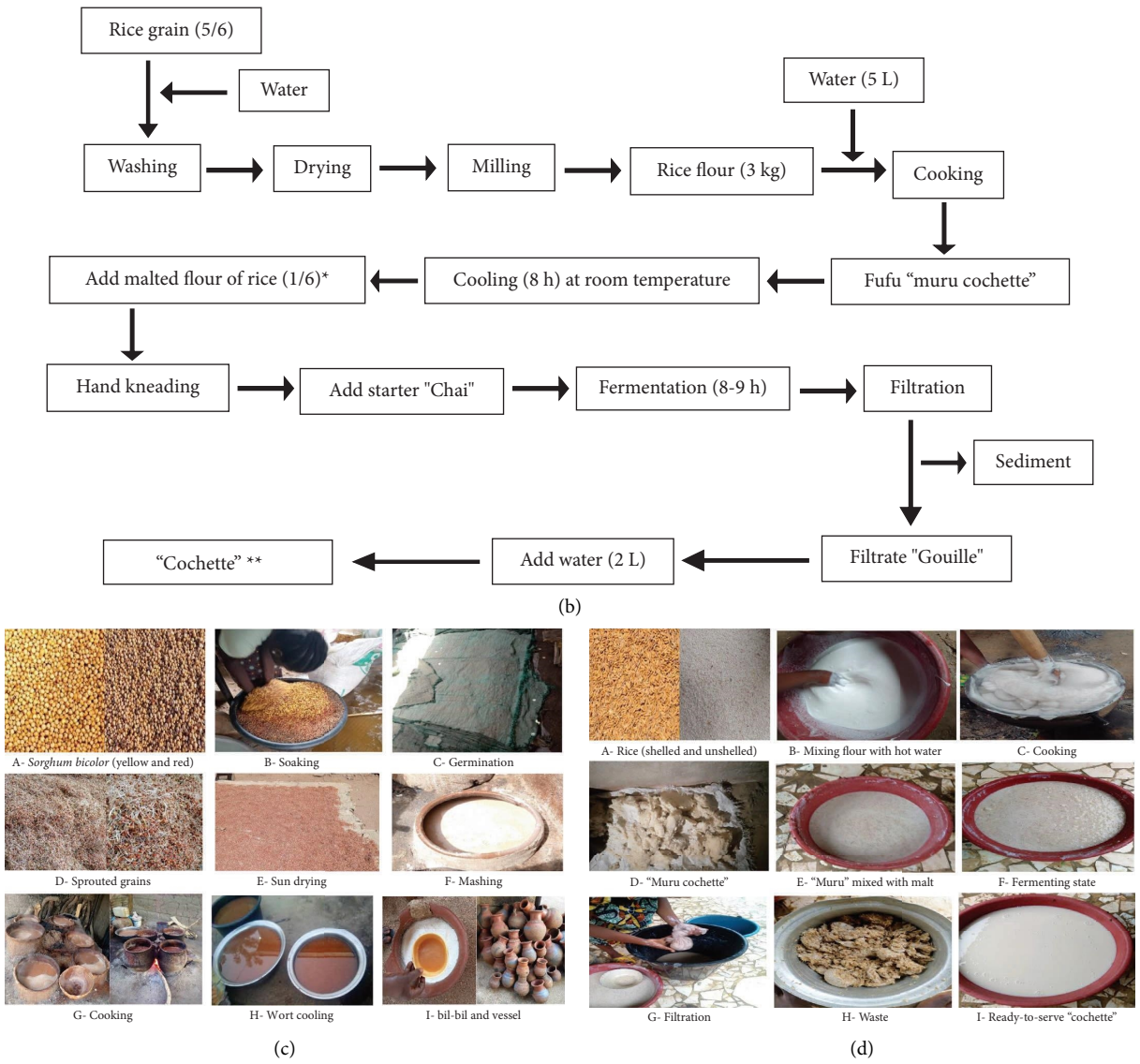


Figure 2: Flow sheet and pictorial showing the traditional processing of the various types of "bili-bili" (a, c) and "cochette" (b, d) by producers in Maroua. *Type of "bili-bili" used for this study. Malted flour of white sorghum can be used and mixed with "muru cochette." **Malted rice "cochette" (from Palar 1) or malted sorghum "cochette" (from Palar 2).

production. After cooking, the obtained sweet wort is decanted, allowed to cool for 5-6 h, and filtered before fermentation. The starter culture also called "chai" is added to the cooled wort and fermented for 9-10 h to produce the final product known as bitter "bili-bili" (Figure 2(c) i). This bitter variant is the most appealed and regarded as the standard "bili-bili." The unique difference between acidic and bitter "bili-bili" processing is the cooling stage, which lasts 13 hours instead of 11 hours.

The customers dislike the acidic variant because it is low alcohol and leads to diarrhoea. The production of sweet and too bitter "bili-bili" differs from bitter "bili-bili" processing at the fermentation stage which was identified by producers as another critical production stage (Table 2). The sweet "bili-bili" is made without the use of "chai" during the fermentation stage. This sweet variant which smells differently causes dysentery and stomach ache when it is consumed. The very bitter "bili-bili" is produced by adding wild "chai" starter during fermentation which lasts 12–14 hours instead of 9-10 hours as during the fermentation of bitter "bili-bili." The consumers dislike the too bitter variant due to headaches caused after its ingestion. The majority of the producers (92.3%) stated that they spend 6-7 days for one run of "bili-bili" production (Table 2). Considering the main production stages, the traditional processing of "bili-bili" is comparable to that of most aforementioned indigenous sorghum-based beers [13, 56–58].

The traditional production of "cochette" is depicted in Figures 2(b) and 2(d). The base cereal used in the production of "cochette" is rice (Table 2). When it is unavailable, white sorghum is used as a substitute (Table 2). According to what

the producers polled, most rice grains (5/6) used to make "cochette" is soaked, dried, and milled. The flour is mixed with boiled water (in a ratio of 3 kg for 5 L) and cooked until the couscous (known as "mourou cochette" in Ngambaye) is obtained (pictorial of Figure 2(d) B–D). After 8 hours of cooling, "mourou cochette" is added to the remaining one-sixth of rice grains malted and milled into flour and the mixture is hand kneaded until full homogenization. If malted rice flour is unavailable, malted sorghum flour can be used. The starter culture known as "chai" is added (sometimes replaced by bitter bili-bili), and the mixture is fermented for 8-9 hours before filtration. The filtrate (called "gouille" in Sara, a Chadian ethnic group) is mixed with about 2 litres of water to produce "cochette." According to Table 2, the majority of the brewers (93.3%) said the production of "cochette" takes 5 days. The production of "cochette" is very similar to that of "zutho," an Indian rice beer [59]. However, the fermentation time differentiates both traditional beers; "zutho" ferments for 2–7 days, whereas "cochette" fermentation takes 8-9 hours. Furthermore, "haria," another rice beer from India, differs from "cochette" in that all rice grains are used unmalted and the mixture is fermented for 3-4 days after the addition of "bakhar" starter culture to the cooked and dried rice [60]. Even though the traditional processing of "cochette" and "mpedli" (a sorghum-based beverage from north Cameroon) is similar, they differ in terms of raw material, starter culture, and fermentation time. In contrast to "cochette," the production of "mpedli" beer utilizes sorghum as the base cereal and its fermentation lasts 2 days without the addition of a starter culture [16].

3.3. Quality Attributes of "Bili-Bili" and "Cochette". The physicochemical profile, proximate composition, and bioactive and antioxidant properties of "bili-bili" and "cochette" marketed in various locations in Maroua are presented in Tables 3 and 4, respectively. The pH values of both traditional beers vary from one locality to another. However, the variations of pH were not statistically different ($p = 0.794$) in "bili-bili" samples but were highly significant ($p \leq 0.001$) in "cochette" samples. The lack of major fluctuations in pH values of "bili-bili" could be attributed to the maturity and mastery of good practices of "bili-bili" producers, who appeared to be more experienced than "cochette" producers. According to the results of field surveys compiled in Table 2, more than half of the "bili-bili" producers (53.8%) were between the ages of 30 and 40 compared to roughly 17% of the "cochette" producers. Furthermore, 46.1% of the "bili-bili" producers had 4–6 years of experience, whilst none of the "cochette" producers had more than 3 years in the field. The lowest mean pH (2.89 ± 0.02) was recorded in "bili-bili" from Domayo and the highest (3.16 ± 0.09) was found in "bili-bili" from Ouro-tchédé. With regards to "cochette" beer, the samples collected from Pitoaré had the lowest pH (3.32 ± 0.05) and those from Palar 2 had the highest average value (4.00 ± 0.03). With pH levels less than 4, both the traditional beers are clearly acidic liquids that aid in the removal of some bacteria. These findings are consistent with prior research, which found acidic pH levels in several traditional cereal-based beers. The pH range of "bili-bili" is close to the values of 2.40–3.26 reported by Ronald and Roger [13] for "red kapsiki" beer but lower than the values of 3.40–3.60, 3.33–3.63, and 3.0–3.8 found in "pito," "tchapalo," and "tchoukoutou," respectively [1]. The pH range of "cochette" comprised the pH values of 3.6 achieved with "zutho" and 3.61–3.66 reported in "haria," two Indian rice beers [59, 60].

The average titratable acidity (TA) value of "bili-bili" samples ranged from 1.09% (Palar and Ouro-tchéde) to 1.17% (Domayo), whereas the mean TA value of "cochette" varied from 0.81 to 0.91% for samples collected from Palar 2 and Pitoaré, respectively. These variations in TA were statistically significant ($p = 0.007$) among the various sampling locations, whereas no significant change ($p = 0.118$) was observed with "cochette" samples from various locations. The range of TA recorded in the current study with "bili-bili" was greater than 0.67–0.81% of "red kapsiki" beer, 0.72–0.96% of "pito" [61], and 0.9–0.99% of "tchapalo" [62]. However, TA values of "cochette" were lower than 1.06–1.42% of "haria" [60] and $1.48\% \pm 0.24$ of "jou" [18]. These variations in TA could be related to the differences in the kind of cereal used as the raw material, microbial activity during the fermentation process, and the fermentation time. The volatile acidity of "bili-bili" (0.741–0.797%) and "cochette" (0.55–0.61%) varied similarly.

The mean temperature values of "bili-bili" samples varied between $37.3 \pm 0.2^{\circ}$C (Palar) and $40.1 \pm 0.5^{\circ}$C (Ouro-tchédé). About "cochette," the lowest average temperature ($38.0 \pm 0.6^{\circ}$C) was noted for the samples collected from Palar 2 and the highest mean value ($39.1 \pm 1.2^{\circ}$C) was recorded for the Pitoaré samples. There were significant differences ($p = 0.044$) in temperatures between the different "bili-bili" samples, but no significant variation ($p = 0.39$) was found across the various samples of "cochette." Temperature affects the growth rate of microorganisms. Temperatures lower than ambient (25–30°C) are beneficial for the microbiological quality of food products. Therefore, high temperatures measured in this study could contribute to an increase in bacterial communities in both traditional beers.

The lowest contents of total soluble solids of "bili-bili" and "cochette" were found in the samples from Domayo ($6.13 \pm 0.11^{\circ}$B) and Palar 1 ($4.06 \pm 0.11^{\circ}$B), respectively. However, the highest mean values were recorded for the samples from Palar ($8.16 \pm 0.85^{\circ}$B) and Palar 2 ($5.03 \pm 0.05^{\circ}$B), respectively. Globally, the soluble solids content of "bili-bili" was higher than that of "cochette." This could be explained why specific gravity (SG) values recorded in "bili-bili" (1.024–1.032) were higher than those of "cochette" (1.015–1.019). "Bili-bili" (3.45 ± 0.07–$4.45 \pm 0.42\%$) had more alcohol content than "cochette" (2.27 ± 0.09–$2.64 \pm 0.04\%$). According to Bayoï and Etoa [20], the alcohol content is affected by both the brix degree and SG. So, the higher the soluble solids and SG, the higher the alcohol content.

The variation in the alcohol content between the two indigenous beers could be attributed to the manufacturing technique. All cereal grains are malted during the "bili-bili" procedure (Table 2), and two cooking stages are observed at

Table 3: Physicochemical profile, proximate composition, and bioactive and antioxidant properties of "bili-bili" samples sold in major production sites of the city of Maroua.

Parameters	Sampling sites				
	Palar ($n = 10$)	Domayo ($n = 10$)	Ouro-tchédée ($n = 10$)	Pont-vert ($n = 10$)	p values*
pH	2.98 ± 0.03^{a}	2.89 ± 0.02^{a}	3.16 ± 0.09^{a}	2.96 ± 0.06^{a}	0.794
Titratable acidity (%, wt/v)	1.09 ± 0.01^{a}	1.17 ± 0.03^{b}	1.090 ± 0.002^{a}	1.10 ± 0.02^{a}	0.007
Volatile acidity (%, wt/v)	0.741 ± 0.003^{a}	0.741 ± 0.003^{a}	0.797 ± 0.024^{b}	0.746 ± 0.001^{a}	0.001
Temperature (°C)	37.3 ± 0.2^{a}	37.8 ± 0.4^{b}	40.1 ± 0.5^{b}	$38.6 \pm 1.9^{a,b}$	0.044
Specific gravity	1.032 ± 0.003^{b}	1.024 ± 0.000^{a}	1.028 ± 0.003^{b}	1.025 ± 0.002^{a}	0.023
Soluble solids content (°B)	8.16 ± 0.85^{b}	6.13 ± 0.11^{a}	$7.06 \pm 0.86^{a,b}$	6.40 ± 0.52^{a}	0.023
Alcohol content (%, v/v)	4.45 ± 0.42^{a}	3.45 ± 0.07^{b}	$3.81 \pm 0.48^{a,b}$	3.53 ± 0.25^{b}	0.030
Electric conductivity (μS/cm)	2179 ± 49^{a}	1880 ± 84^{b}	2061 ± 51^{a}	2127 ± 6^{a}	0.000
Dissolved solids content (ppm)	1091.3 ± 26.2^{a}	939.0 ± 40.9^{b}	1047.3 ± 10.9^{c}	1057.0 ± 9.7^{c}	0.000
Dry matter (%, wt/wt)	7.10 ± 1.06^{a}	5.43 ± 0.02^{b}	9.51 ± 1.15^{a}	3.41 ± 1.23^{c}	0.026
Total soluble protein (g/100 mL)	0.489 ± 0.005^{a}	0.615 ± 0.003^{b}	0.551 ± 0.003^{c}	0.516 ± 0.006^{d}	0.000
Soluble amino acid (g/100 mL)	0.170 ± 0.004^{a}	0.420 ± 0.003^{b}	0.380 ± 0.004^{c}	0.245 ± 0.006^{d}	0.000
Total carbohydrates (g/100 mL)	0.614 ± 0.002^{a}	0.467 ± 0.004^{b}	0.397 ± 0.002^{c}	0.607 ± 0.002^{d}	0.000
Reducing sugars (g/100 mL)	0.307 ± 0.049^{a}	0.260 ± 0.006^{a}	0.248 ± 0.001^{a}	0.328 ± 0.005^{a}	0.086
Ash content (%, m/m)	0.50 ± 0.33^{a}	1.59 ± 0.84^{a}	1.23 ± 0.67^{a}	2.74 ± 1.27^{a}	0.350
α-Carotene content (mg/100 mL)	108.5 ± 5.2^{a}	87 ± 4.7^{b}	98.4 ± 4.7^{c}	82.3 ± 3.1^{b}	0.015
β-Carotene content (mg/100 mL)	129.8 ± 1.1^{a}	102.8 ± 8.3^{b}	98.3 ± 5.5^{b}	99.9 ± 0.0^{b}	0.009
Lycopene content (mg/100 mL)	80.7 ± 1.2^{a}	78.9 ± 3.7^{a}	69.4 ± 4.5^{a}	68.0 ± 3.3^{a}	0.050
Lutein content (mg/100 mL)	98.0 ± 2.8^{a}	101.6 ± 4.5^{a}	101.2 ± 7.3^{a}	121.2 ± 5.0^{b}	0.034
Tannin content (mgCE/100 mL)	273.3 ± 2.6^{a}	250.7 ± 2.0^{b}	266.6 ± 1.5^{c}	245 ± 4.55^{b}	0.001
Polyphenols (mgGAE/100 mL)	410.7 ± 1.3^{a}	230.7 ± 3.1^{b}	296.5 ± 2.2^{c}	364.3 ± 1.3^{d}	0.000
Total flavonoids (mgQE/100 mL)	360.9 ± 1.9^{a}	203.4 ± 2.8^{b}	349.3 ± 1.9^{c}	343.9 ± 2.8^{c}	0.000
DPPH scavenging activity (%)	48.37 ± 0.12^{a}	30.47 ± 1.15^{b}	45.56 ± 0.25^{c}	45.02 ± 1.02^{c}	0.000
DPPH antioxidant capacity (mgTE/100 mL)	56.1 ± 0.1^{a}	33.1 ± 1.4^{b}	51.8 ± 1.3^{c}	52.5 ± 0.3^{c}	0.000
Ferric antioxidant power (mgTE/100 mL)	122.9 ± 0.7^{a}	95.4 ± 0.7^{b}	73.3 ± 0.7^{c}	57.7 ± 0.5^{d}	0.000
PAC_{DPPH}	0.14 ± 0.00^{a}	0.13 ± 0.00^{a}	0.17 ± 0.00^{a}	0.14 ± 0.00^{a}	0.070
PAC_{FRAP}	0.3 ± 0.00^{a}	0.46 ± 0.00^{b}	0.25 ± 0.00^{a}	0.16 ± 0.00^{c}	0.043
RACI	0.96 ± 0.25^{a}	-0.97 ± 0.84^{a}	-0.03 ± 0.46^{a}	0.04 ± 0.73^{a}	0.814

* p values lower than 0.05 indicate significant differences in mean values of samples from the different locations. Two or more mean values with the same lower case superscript letters are not significantly different ($p > 0.05$) in the same row. PAC_{DPPH} and PAC_{FRAP}: phenolic antioxidant capacity based on DPPH and FRAP assays; RACI: relative antioxidant capacity index.

Table 4: Physicochemical profile, proximate composition, and bioactive and antioxidant properties of "cochette" samples collected in different marketing places of Maroua city.

Parameters	Sampling sites			
	Palar 1 ($n = 12$)	Palar 2 ($n = 12$)	Pitoare ($n = 16$)	p values*
pH	3.43 ± 0.02^{a}	4.00 ± 0.03^{b}	3.32 ± 0.05^{c}	0.000
Titratable acidity (%, wt/v)	0.83 ± 0.03^{a}	0.81 ± 0.01^{a}	0.91 ± 0.08^{a}	0.118
Volatile acidity (%, wt/v)	0.55 ± 0.02^{a}	0.55 ± 0.01^{a}	0.61 ± 0.05^{a}	0.118
Temperature (°C)	38.8 ± 0.7^{a}	38.0 ± 0.6^{a}	39.1 ± 1.2^{a}	0.390
Specific gravity	1.015 ± 0.000^{a}	1.019 ± 0.000^{b}	1.018 ± 0.000^{b}	0.000
Soluble solids content (°B)	4.06 ± 0.11^{a}	5.03 ± 0.05^{b}	4.73 ± 0.23^{c}	0.000
Alcohol content (%, v/v)	2.27 ± 0.09^{a}	2.64 ± 0.04^{b}	2.52 ± 0.11^{b}	0.005
Electric conductivity (μS/cm)	1064 ± 33^{a}	915 ± 0^{b}	1697 ± 0^{c}	0.000
Dissolved solids content (ppm)	539 ± 28^{a}	457 ± 0^{b}	848 ± 0^{c}	0.000
Dry matter (%, wt/wt)	12.03 ± 1.26^{a}	11.80 ± 1.60^{a}	10.12 ± 0.19^{a}	0.348
Total soluble protein (g/100 mL)	0.752 ± 0.003^{a}	0.713 ± 0.005^{b}	0.681 ± 0.008^{c}	0.001
Total soluble amino acid (g/100 mL)	0.491 ± 0.004^{a}	0.385 ± 0.004^{b}	0.252 ± 0.006^{c}	0.000
Total carbohydrate (g/100 mL)	0.554 ± 0.007^{a}	0.504 ± 0.004^{b}	0.459 ± 0.004^{c}	0.001
Reducing sugar (g/100 mL)	0.253 ± 0.007^{a}	0.200 ± 0.002^{b}	0.171 ± 0.004^{c}	0.001
Ash content (%, m/m)	0.487 ± 0.120^{a}	0.508 ± 0.019^{a}	0.522 ± 0.045^{a}	0.174
α-Carotene content (mg/100 mL)	67.7 ± 0.5^{a}	77.8 ± 1.1^{b}	72.2 ± 2.6^{a}	0.020
β-Carotene content (mg/100 mL)	95.6 ± 1.6^{a}	94.8 ± 0.5^{a}	92.8 ± 3.3^{a}	0.510
Lycopene content (mg/100 mL)	73.0 ± 1.2^{a}	104.0 ± 0.8^{b}	74.8 ± 0.4^{a}	0.0000
Lutein content (mg/100 mL)	111.6 ± 1.6^{a}	109.2 ± 2.8^{a}	99.2 ± 1.1^{b}	0.016
Polyphenols (mgGAE/100 mL)	252.3 ± 2.7^{a}	462.3 ± 3.1^{b}	342.7 ± 0.9^{c}	0.0000

Table 4: Continued.

Parameters	Sampling sites			p values*
	Palar 1 ($n = 12$)	Palar 2 ($n = 12$)	Pitoare ($n = 16$)	
Total flavonoids (mgQE/100 mL)	280.9 ± 0.9^a	296.8 ± 1.9^b	258.9 ± 4.8^c	0.0000
Tannin content (mgCE/100 mL)	230.0 ± 4.1^a	100.0 ± 1.5^b	266.2 ± 3.1^c	0.0000
DPPH scavenging activity (%)	26.7 ± 1.2^a	51.7 ± 1.7^b	31.6 ± 1.2^c	0.0004
Scavenging capacity (mgTE/100 mL)	30.6 ± 1.6^a	60.4 ± 2.3^b	39.6 ± 1.6^c	0.0004
Reducing power (mgTE/100 mL)	138.3 ± 0.0^a	129.7 ± 0.9^b	90.6 ± 0.3^c	0.0000
PAC_{DPPH}	0.12 ± 0.00^a	0.13 ± 0.00^a	0.11 ± 0.00^a	0.310
PAC_{FRAP}	0.55 ± 0.01^a	0.28 ± 0.00^b	0.26 ± 0.00^b	0.034
RACI	-0.48 ± 0.81^a	0.90 ± 0.34^a	-0.42 ± 0.50^a	0.610

p values* lower than 0.05 indicate significant differences in mean values of samples among the different locations. Two or more mean values with the same lower case superscript letters are not significantly different (>0.05) in the same row. PAC_{DPPH} and PAC_{FRAP}: phenolic antioxidant capacity based on DPPH and FRAP assays; RACI: relative antioxidant capacity index.

the brewing stage (Figure 2), resulting in an increase in sugars available for alcohol conversion during fermentation. In contrast, just a small portion of the cereal used in "cochette" production is malted, and there is a single cooking stage. Both opaque beers had a lower alcohol content than "dora-bonga" (3.94–4.66%), "tchapalo" (5.08–5.22%), "haria" (3.4–11%), and "jou" (5.30–22.05%) [18, 57, 60, 62]. Therefore, due to its low alcohol content compared to "bili-bili" and the previously studied African beers, "cochette" can be consumed in adequate quantities as a refreshing drink to compensate the body water loss during the hot summer season in northern Cameroon.

The mean EC and TDS values of "bili-bili" (1880–2179 μS/cm and 939.0–1091.3 ppm, respectively) were greater ($p \leq 0.001$) than those of "cochette" (915–1697 μS/cm and 457–848 ppm, respectively). The difference in EC and TDS between "bili-bili" and "cochette" could be attributed to the mineral salts, organic acids, and proteins content of the raw materials used in the production. The EC is a parameter used to determine the ionisation degree of a sample by measuring the concentration of mineral ions and ionizable compounds. Given that the EC values of both traditional beers are higher than 1000 μS/cm, they can be considered highly mineralized.

Changes in the dry matter were statistically significant in "bili-bili" ($p = 0.026$) and not significant in "cochette" ($p = 0.348$) among the various sampling sites. The highest mean dry matter (DM) for "bili-bili" and "cochette" was recorded in the samples collected from Ouro-tchédé ($9.51 \pm 1.15\%$) and Palar 1 ($12.03 \pm 1.26\%$), respectively, while the lowest means were recorded in samples from Pont-vert ($3.41 \pm 1.23\%$) and Pitoaré ($10.12 \pm 0.19\%$), respectively. The dry matter of both cereal-based beers measured in this investigation was within the 5–13% range as previously reported for various African beers [63]. However, the DM contents of "bili-bili" and "cochette" were lower than the values of 15.4–20.2% found in "tchoukoutou/chakpalo" [64]. These variations might be associated with the volume of water added, the cereal used, and time required to ferment the wort.

Total soluble protein and amino acid changed significantly ($p \leq 0.001$) in "bili-bili" and "cochette" samples. The average soluble protein and amino acid levels (g/100 mL) of the "bili-bili" samples varied between 0.489 and 0.615 and 0.170 and 0.420, respectively. Both parameters for "cochette" samples ranged from 0.681 to 0.752 and from 0.252 to 0.491, respectively. The protein levels recorded in this study were higher than 0.14–0.39 g/100 mL reported in "pito" [61] and 1.1–6.5 mg/100 mL reported in "dolo" [65], but lower than those reported in Central African Republic for "bili-bili" (2.79–2.90%) and "dora-bonga" (3.80–3.82%) [57]. Because of their relatively high protein content, "bili-bili" and "cochette" could be viewed as additional meals used to supplement the protein-demand gap for the consumers and families who cannot afford more expensive animal-based proteins. The amino acid levels of both beers were similar to 0.283–0.381 g/100 mL as previously reported for "kounou," a fermented cereal beverage [15]. Therefore, "bili-bili" and "cochette" intake may also be a rich source of essential amino acids important for the human diet.

Changes in total carbohydrates were significant ($p \leq 0.001$) in "bili-bili" and "cochette." However, changes in reducing sugars were only significant ($p = 0.001$) in "bili-bili" samples. The highest total sugar and reducing sugar levels were found in the "bili-bili" samples from Palar (0.614 g/100 mL) and Pont-vert (0.328 g/100 mL), respectively, as well as the "cochette" samples from Palar 1 (0.554 and 0.253 g/100 mL, respectively). These results were higher than those obtained in "dolo" (1.1–8.4 mg/100 mL and 4.21–19.94 μg/mL, respectively). On the contrary, total sugar contents were lower than those recorded in "dora-bonga" (0.65–0.67%), "pito" (0.86–2.35 g/100 mL), "red kapsiki" beer (41.8–72.9 g/L), and "tchapalo" (8.84 g/100 mL). These variations could be explained by the sugar content of the wort and its fermentation degree. It is well-known that the more the beer ferments, the less the sugar remains. Sugars are a good source of energy for the body; so, drinking both the cereal-based beers ("bili-bili" and "cochette") will assist customers to achieve their energy needs. This is why "bili-bili" and "cochette" are widely referred to as "manger-boire" drinks as many African fermented beverages because they are regarded both as a meal to satisfy hunger and a drink to relieve thirst.

The ash content varied nonsignificantly ($p = 0.174$) between $0.50 \pm 0.33\%$ and $2.74 \pm 1.27\%$ for the "bili-bili" samples from Palar and Pont-vert, respectively. The lowest average ash content was found in the "cochette" samples from Palar 1 ($0.487 \pm 0.120\%$) and the highest mean value was recorded in the samples from Pitoaré ($0.522 \pm 0.045\%$). The ash contents of both the traditional beers were higher than those recorded in "pito" (0.001%), "kounou"

(0.24 ± 0.13%), "tchapalo" (0.27%), and "aliha" (0.09–0.19%). This indicates that "bili-bili" and "cochette" may provide more minerals to consumers than aforementioned fermented cereal-based drinks.

The carotenoid contents (mg/100 mL) of "bili-bili" and "cochette" samples were measured and expressed as α-carotene, β-carotene, lycopene, and lutein equivalents (Tables 3 and 4). "Bili-bili" samples from Palar had the highest α-carotene, β-carotene, and lycopene equivalents (108.5 ± 5.2, 129.8 ± 1.1, and 80.7 ± 1.2, respectively), while the samples from Pont-vert had the highest lutein (121.2 ± 5.0) and the lowest α-carotene, β-carotene, lycopene equivalents (82.3 ± 3.1, 99.9 ± 0.0, and 68.0 ± 3.3, respectively). The "cochette" samples collected from Palar 2 had the highest α-carotene and lycopene equivalents (77.8 ± 1.1 and 104.0 ± 0.8, respectively), while Palar 1 had the highest β-carotene and lutein equivalents (95.6 ± 1.6 and 111.6 ± 1.6, respectively). These variations in the carotenoids composition are caused by a number of reasons, including the origin and degree of coloring of the raw materials employed and the diluting effect of water. The geographic origin of the sample should be ruled out because the climatic conditions applied in all the sampling sites were similar. Many studies revealed that carotenoids may protect against cancer [66], cardiovascular diseases [67], and aging-related degenerative disorders [68]. Therefore, both traditional beers may be a rich source of natural carotenoids and their intake may benefit to the consumers' health.

In the current investigation, significant differences ($p \leq 0.001$) in phenolic, flavonoids, and tannin contents were found in "bili-bili" and "cochette" samples collected from different locations. The mean total phenolic content (mgGAE/100 mL) of "bili-bili" ranged from 230.7 ± 3.1 (Domayo samples) to 410.7 ± 1.3 (Palar samples). The TPC of "bili-bili" in the current study is more than 38.48–71.062 mg GAE/100 mL found by Charles et al. [51] for "bili-bili" and the 84.3–115 mg/100 mL reported by Ronald and Roger [13] for red kapsiki beer, both processed in far north Cameroon. The highest mean TPC of "cochette" was found in Palar 2 samples (462.3 ± 3.1), while the lowest was found in Palar 1 (252.3 ± 2.7 mg GAE/100 mL). The average TPC of "cochette" was higher than the 12.8–63.13 mg GAE/100 mL reported by Handique et al. [69] for traditional Indian rice beers. These differences in the TPC could be due to variances in processing procedures as well as the protein and sugar levels of the beverage. The TPC is determined using the Folin–Ciocalteu reagent known to react with the phenolic groups and with sugars, proteins, and organic acids which may interfere with the final result. Given that the TPC of both traditional beers exceeds 100 mg GAE/100 mL [70], they may be considered as an important source of phenolic compounds, which are known as human health-promoting molecules.

The flavonoid contents (mg QE/100 mL) of "bili-bili" and "cochette" ranged from 203.4 to 360.9 and 258.9 to 296.8, respectively. These levels were lower than those found in "jou" (10.04 ± 0.29 mgQE/mL) and "kounou" (1660 ± 190 mgQE/100 mL), two cereal-fermented drinks from India and Cameroon, respectively.

The lowest and highest condensed tannin levels (mg CE/100 mL) of "bili-bili" were recorded in samples from Pont-vert (245 ± 4.55) and Palar (273.3 ± 2.6), respectively. "Cochette" recorded average condensed tannin contents ranging from 100.0 ± 1.5 to 266.2 ± 3.1 mg CE/100 for the samples from Palar 2 and Pitoaré, respectively. Tannins, bitter and astringent compounds, are antinutrients that can reduce protein digestibility and mineral absorption and impede digestive enzymes. It has been observed that tannin contents ranging from 108.3 to 120 mg/kg are high enough to pose a health risk [71]. Given that the tannin levels recorded in the current study are significantly higher than the stated values, additional efforts to minimise tannins in both traditional beers are required to prevent the related health concerns. Therefore, the moderate intake of both opaque beers may contribute to promote human health due to the presence of carotenoids, polyphenols, flavonoids, and tannins, known as antioxidant molecules.

The antioxidant activity of a sample depends on various parameters which are not able to be reported by a single method. So, the DPPH and FRAP assays were used to determine antioxidant properties of "bili-bili" and "cochette" in terms of the radical-scavenging activity and ferric reducing power, respectively. Both parameters changed significantly ($p \leq 0.001$) in "bili-bili" and "cochette" samples from various locations. "Bili-bili" from Palar (48.37 ± 0.12%; 56.1 ± 0.1 mgTE/100 mL) and "cochette" from Palar 2 (51.7 ± 1.7%; 60.4 ± 2.3 mgTE/100 mL) possessed the maximum radical-scavenging activity. "Bili-bili" samples from Domayo (30.47 ± 1.15%; 33.1 ± 1.4 mgTE/100 mL) and "cochette" from Palar 1 (26.7 ± 1.2%; 30.6 ± 1.6 mgTE/100 mL) recorded the lowest DPPH-scavenging activity. The free radical-scavenging activity of "bili-bili" was lower than that registered with "kounou" (61.93 ± 6.94%; 74.09 ± 6.62 mgTE/100 mL). However, the scavenging activity previously found in fresh "jou" (39.96%) was within the range of values recorded with "cochette." The ferric-reducing antioxidant power (mgTE/100 mL) of "bili-bili" and "cochette" ranged from 57.7 ± 0.5 (Pont-vert) to 122.9 ± 0.7 (Palar) and from 90.6 ± 0.3 (Pitoaré) to 138.3 ± 0.0 (Palar 1), respectively. Both cereal-fermented beverages had the reducing power values lower than that of 791 ± 55.4 mgTE/100 mL of kounou reported by Bayoï et al. [15]. Changes in the antioxidant activity between both traditional beers could be attributed to differences in raw materials, production process, and fermentation conditions.

The phenolic antioxidant capacity (PAC) enables the comparison of the effectiveness of phenolic compounds present in the tested samples and provides specific insight into the applied assay. Globally, the PAC of the various local beer samples was lower than 1. This suggests that phenolic compounds played a minor role in the scavenging activity and reducing power of both traditional beverages. The highest PAC values were recorded in "bili-bili" collected from Ouro-tchédé ($PAC_{DPPH} = 0.17 \pm 0.00$) and "cochette" from Palar 1 ($PAC_{FRAP} = 0.55 \pm 0.01$).

Given the limitations of each approach for measuring the antioxidant activity, the relative antioxidant capacity index (RACI) method provides an alternate method which integrates several antioxidant chemical analyses. In this study,

values from DPPH, FRAP, and TPC assays were used to generate the RACI value, which is a unitless scientific value used to rank the antioxidant activity of foods. There was no significant change ($p = 0.814$) in the RACI values between the various "bili-bili" samples. Palar samples had the highest mean RACI value (0.96) followed by Pont-vert (0.04), Ouro-tchédé (−0.03), and Domayo (−0.97). The RACI values of "cochette" samples varied nonsignificantly ($p = 0.61$) between -0.48 ± 0.81 and 0.90 ± 0.34. Palar 2 samples had the highest RACI value (0.90) followed by those from Pitoaré and Palar 1 which had RACI values of −0.42 and −0.48, respectively. Given the scarcity of research using the RACI to evaluate the antioxidant activity of food products, the current work is one of the rare studies using the RACI to compare the food antioxidant activity, so the values recorded in this study cannot be compared to those in the literature. However, the RACI values of "bili-bili" were found to be higher than those of "cochette." So, the antioxidant activity of "bili-bili" appears to be greater than that of "cochette." Based on this, "bili-bili" consumption may be more appropriate for mitigating oxidative stress-related disorders.

3.4. Microbiological Quality of "Bili-Bili" and "Cochette". The mean counts of microbial groups enumerated in "bili-bili" and "cochette" samples from various localities in Maroua city are presented in Table 5. Total aerobic mesophilic bacteria (TAMB), total coliforms (TCs), fecal coliforms (FCs), total aerobic and mesophilic spore-forming bacteria (TAMSFB), and sulphite-reducing *Clostridia* (SRC) were counted in all the samples of both traditional beers, except the "bili-bili" samples from Palar and Domayo in which no fecal coliform was detected. The enumeration of all the microbial groups detected in "bili-bili" varied significantly ($p < 0.05$) among the samples from the various locations. In contrast, most microbial groups enumerated in the "cochette" samples varied nonsignificantly ($p > 0.05$) from one sampling site to another. However, sole FC and SRC groups changed significantly ($p < 0.01$) in the "cochette" samples. All enumerated microbial groups were found above the limits applied to each group. The minimum and maximum TAMB counts in "bili-bili" were recorded in the samples from Palar (1.5×10^{11} cfu/mL) and Domayo (2×10^{11} cfu/mL), respectively. TAMB counts of "cochette" ranged from $(1.10 \pm 0.42)10^{10}$ to $(1.50 \pm 0.70)10^{10}$ cfu/mL in samples collected from Palar 2 and Palar 1, respectively. TAMB counts of both traditional beers were higher than 3.18–4.58 log and 5.98 ± 0.44 log reported, respectively, in "shameta" and "borde," two Ethiopian maize-based fermented beverages [17, 72]. Known as the quality indicator, the high count in TAMB may be related to the poor hygiene of the production site and sale point, as well as the utensils used during the processing and serving. Furthermore, this could be linked to the temperature of beer samples ranged from 30 to 40°C required for the optimal growth of bacteria and other mesophilic microorganisms.

The lowest TC and TF counts (cfu/mL) were found in "bili-bili" samples from Ouro-tchédé (1.1×10^6) and Pont-vert (2×10^8), respectively. Meanwhile, the highest counts were recorded in the samples from Domayo (9.75×10^6 and 1.50×10^{10}, respectively). The highest FC counts were found in "bili-bili" samples from Ouro-tchédé (6.5×10^2) and Pont-vert (1.5×10^3). The maximum values (cfu/mL) of the three microbial groups were recorded in the samples of "cochette" from Palar 2 (1.8×10^7, 7.5×10^7, and 8.4×10^3, respectively). TC, FC, and TF counts found in the current study were in a higher range than those reported with "kounou" [15]. The presence of coliforms in beers may be due to unsanitary practices during handling, transport, and sale. Fecal coliforms in both the fermented beverages could be explained by a postfermentation exogenous fecal contamination from environment, utensils, water, and handling, whereas the presence of fungi may be related to the very acidic pH (less than 4) and high-water content of both cereal-fermented beverages. The presence of fungi in both beers is a call for concern given that they are cereal-based beverages. Indeed, cereals are frequently contaminated by a variety of fungi (*Aspergillus*, *Fusarium*, *Penicillium,* etc.) known to produce mycotoxins causing numerous foodborne diseases in consumers [73]. Mycotoxins such as aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone are the majorly identified in locally fermented cereal-based beverages. It has been reported that drinking of traditional beer increased exposure to some of the aforementioned mycotoxins by at least 6-fold above the recommended levels [74].

TAMSFB and SRC counts (cfu/mL) in "bili-bili" samples were lowest in Domayo (1.6×10^6 and 1.4×10^2, respectively) and highest in Pont-vert (3.1×10^6 and 4.7×10^2, respectively). The "cochette" samples from Palar 1 and Pitoaré had the lowest counts of both microbial groups (1.1×10^6 and 2.2×10^2, respectively), whereas the samples from Palar 2 had the greatest counts (2.2×10^7 and 8.5×10^2, respectively). TAMSFB and SRC counts of both beers were greater in the current study than those reported in "shameta" [17] and "kounou" [75], respectively. The presence of aerobic and mesophilic spore-forming bacteria in both beers could be used as an indicator of *Bacillus* spp., which is one of the troublesome food contaminants due to their ability to produce toxins and endospores [76]. The source of this microbial flora could be the raw materials and utensils employed during the production. Furthermore, aerobic spore-forming bacteria are amylolytic bacteria which can produce amylases and proteases that stimulate the growth of other microbial groups and product deterioration [17]. Because sulphite-reducing bacteria (SRC) are an indication of soil contamination, their presence in both the traditional beers may suggest the use of either raw materials or utensils contaminated, either the contact of the product or one of its intermediates with the soil. Furthermore, the presence of SRC presumes that of *Clostridium perfringens*, frequently implicated in food poisoning cases.

No fecal *Streptococci*, *Salmonella*/*Shigella*, or *Staphylococci* spp. was found in any of the "bili-bili" and "cochette" samples. Both traditional beers were pathogen free due to their extremely acidic pH and the presence of endogenous antimicrobial compounds such as alcohol, phenolic compounds, and carotenoids. The absence of fecal *Streptococci* and *Staphylococci* spp. adds to our understanding of the

Table 5: Mean microbial loads of different microbial groups in "bili-bili" and "cochette" samples from various selling places of Maroua city.

Microbial groups (cfu/mL)	Sampling sites (bili-bili)					
	Palar ($n = 10$)	Domayo ($n = 10$)	Ouro-tchede ($n = 10$)	Pont-vert ($n = 10$)	p values*	Standard
Total aerobic mesophilic bacteria	$(1.50 \pm 0.07)10^{11a}$	$(2.00 \pm 0.05)10^{11b}$	$(1.70 \pm 0.04)10^{11c}$	$(1.90 \pm 0.09)10^{11b}$	0.016	$<10^6$
Total coliforms	$(3.20 \pm 0.35)10^{6a}$	$(9.75 \pm 1.06)10^{6b}$	$(1.1 \pm 0.14)10^{6c}$	$(5.95 \pm 3.48)10^{6d}$	0.000	$<10^3$
Fecal coliforms	Nil	Nil	$(6.50 \pm 2.12)10^{2b}$	$(1.5 \pm 0.21)10^{3c}$	0.002	$<10^2$
Total fungi	$(2.50 \pm 0.70)10^{8a}$	$(1.50 \pm 0.63)10^{10b}$	$(2.50 \pm 0.53)10^{8a}$	$(2.00 \pm 0.01)10^{8a}$	0.019	$<10^5$
Total aerobic and mesophilic spore-forming bacteria	$(2.10 \pm 0.70)10^{6a}$	$(1.60 \pm 0.13)10^{6a}$	$(1.90 \pm 0.35)10^{6a}$	$(3.10 \pm 0.28)10^{6b}$	0.046	$<10^4$
Fecal *Streptococci*	Nil	Nil	Nil	Nil	—	$<10^3$
Sulphite reducing *Clostridia*	$(2.25 \pm 0.35)10^{2a}$	$(1.40 \pm 0.14)10^{2b}$	$(3.40 \pm 0.56)10^{2c}$	$(4.70 \pm 0.28)10^{2d}$	0.003	$<10^5$
Salmonella/*Shigella*	Nil	Nil	Nil	Nil	—	0/20 g
Staphylococci spp.	Nil	Nil	Nil	Nil	—	$<10^2$
Microbial groups (cfu/mL)	Sampling sites (cochette)					
	Palar 1 ($n = 12$)	Palar 2 ($n = 12$)	Pitoare ($n = 16$)	p values*	Standard	
Total aerobic mesophilic bacteria	$(1.50 \pm 0.70)10^{10a}$	$(1.10 \pm 0.42)10^{10a}$	$(1.20 \pm 0.28)10^{10a}$	0.734	$<10^6$	
Total coliforms	$(1.90 \pm 0.21)10^{6a}$	$(1.80 \pm 0.50)10^{7a}$	$(1.00 \pm 0.63)10^{7a}$	0.256	$<10^3$	
Fecal coliforms	$(3.20 \pm 0.14)10^{3a}$	$(8.40 \pm 0.84)10^{3b}$	$(5.40 \pm 0.56)10^{3a}$	0.007	$<10^2$	
Total fungi	$(7.50 \pm 0.70)10^{7a}$	$(7.50 \pm 2.12)10^{7a}$	$(7.00 \pm 1.43)10^{7a}$	0.932	$<10^5$	
Total mesophilic spore-forming bacteria	$(1.10 \pm 0.21)10^{6a}$	$(2.20 \pm 1.01)10^{7a}$	$(1.50 \pm 0.70)10^{6a}$	0.055	$<10^4$	
Fecal *Streptococci*	Nil	Nil	Nil	—	$<10^3$	
Sulphite-reducing *Clostridia*	$(2.80 \pm 0.28)10^{2a}$	$(8.50 \pm 0.70)10^{2b}$	$(2.20 \pm 0.38)10^{2a}$	0.001	$<10^5$	
Salmonella/*Shigella*	Nil	Nil	Nil	—	0/20 g	
Staphylococci spp.	Nil	Nil	Nil	—	$<10^2$	

Two or more mean microbial values with the same lower case superscript letters are not significantly different ($p > 0.05$) in the same row. p values* lower than 0.05 indicate significant differences in mean values of samples among the different locations.

suspected fecal contamination. Indeed, fecal *Streptococci* and sulphite-reducing bacteria (SRC) provide temporal information about the contamination, whereas *Staphylococci* spp. indicates that the contamination was caused by humans. Given the presence of SRC and the absence of *Staphylococci* in the samples, the apparent fecal contamination described in this study could be old but not of human origin. However, considering that the fecal coliforms/total coliforms ratio is less than 1, this could point to an animal origin for the suspected fecal contamination.

3.5. Sensory Properties. Sensory profiles of "bili-bili" and "cochette" from various areas in Maroua are summarized in Table 6. Except for "bili-bili" which had a substantial bitterness, there was no significant difference ($p > 0.05$) in most sensory attributes of these traditional beers. These findings were consistent with those previously reported by Bayoï et al. [14, 15]. As with other local beverages, Bayoï et al. [14] observed that color was the unique attribute that fluctuated considerably during the sensory examination of "téa lémi," a traditional pummelo wine from the far north of Cameroon. On the contrary, Bayoï et al. [15] showed that the provenance of samples had no effect on the sensory characteristics of "kounou." The homogeneity of "cochette" sensory attributes suggests the mastery of the production scheme and the best reflex which should be operated by the producers to ensure a regular sensory quality. Another reason could be the origin of "cochette" producers. Indeed, "cochette" is traditional rice beer brewed solely by Chadian women, while "bili-bili" is produced by women from Cameroon, Tchad, and the Central African Republic (Table 2).

"Bili-bili" samples from Pont-vert recorded the best score in terms of bitterness (7.0 ± 1.8), while those from Palar had the best mean scores in terms of flavor/odor (7.4 ± 1.7) and color (7.5 ± 2.2). The highest mean scores of alcoholic taste, texture, and overall acceptability were received with the samples from Ouro-tchédé (6.5 ± 1.9, 7.3 ± 1.9, and 7.5 ± 1.1, respectively). Alcoholic taste (6.3 ± 2.3) and color (7.2 ± 1.7) were the major sensory attributes for the acceptance of "cochette" collected in Palar 1 and Palar 2, respectively. "Cochette" from Pitoaré received the highest average scores of 6.3 ± 2.6, 6.4 ± 1.7, 7.0 ± 1.5, and 6.9 ± 1.5 in terms of bitterness, texture, flavor/odor, and overall acceptability, respectively. Therefore, "cochette" from Pitoaré appears to be more popular among the panellists than "cochette" from Palar 1 and Palar 2. This could be explained by the fact that "cochette" is only produced from rice in Pitoaré (major method), whereas this local beer is prepared from rice or sorghum in Palar locations (minor technique).

3.6. Safety Status of "Bili-Bili" and "Cochette". The safety scores of "bili-bili" and "cochette" marketed in various Maroua areas are presented in Table 7. Assessment of the safety status of "bili-bili" and "cochette" was carried out using the safety scores calculated by integrating the microbial scores assigned according to the microbial counts of each sample. The safety level of the sample was defined as follows: the lower the safety score (0–45-point scale), the better the safety status of sample. Therefore, based on the safety scores of "bili-bili" samples, the safety status can be classified as follows: Palar and Domayo (139.5) > Ouro-tchédé (139.9) > Pont-vert (141). The safety status of the "cochette" samples ranged as follows: Palar 1 and Pitoaré (96.5) are superior to Palar 2 (141). As indicated in Table 7, the safety scores of all the collected samples were greater than 45, suggesting they were potentially dangerous for the consumer's health. Based on the overall safety scores received by the two traditional cereal-based beers, "bili-bili" (559.5) may be considered more dangerous than "cochette" (334). It is crucial to note, however, that the number of localities chosen (4) for assessing the safety status of "bili-bili" was higher than that considered (3) for analysing the safety status of "cochette." Therefore, this may illegitimate comparing of the safety status of the two types of traditional beers marketed in the city of Maroua.

3.7. Correlation Analysis. Heat maps Pearson correlation matrices of the physicochemical, proximate, sensory, microbiological properties, phenolic compounds, and antioxidant activities of "bili-bili" and "cochette" are plotted in Figure 3. Alcohol and TSS were positively correlated in "bili-bili" ($r = 0.995$) and "cochette" ($r = 1.000$). This indicates that alcohol as the water-soluble molecule might be accounted for the soluble solids content of both opaque beers. Furthermore, negative and significant correlations were found between pH and titratable acidity ($r = -0.673$ and $r = -0.742$, respectively), suggesting that the acidic pH values express high organic acids content in "bili-bili" and "cochette."

With regards to "bili-bili," the protein content was negatively correlated to the electrical conductivity ($r = -0.994$) and TPC ($r = -0.988$). This suggests that the proteins in "bili-bili" were less electrically charged and more neutral. Given the acidic pH of this traditional beer, this may imply that the proteins in "bili-bili" are rich in amino acids, normally nonionised and neutral at very low pH. Furthermore, the negative correlation between proteins and the TPC insinuates the low level of "bili-bili" proteins in hydroxylated amino acids, especially tyrosine (known as phenolic amino acid).

The TFC and DPPH were inversely associated to the titratable acidity ($r = -0.997$ and $r = -0.990$, respectively), implying that "bili-bili" with low acidity levels had the best bioactive properties. This correlation is highly interesting because the negative health effect of strong acidity (gastric ulcers and bone and teeth erosion) could be countered by flavonoids, which act as a guardian against inflammation and oxidative damage. This was verified by a high positive correlation between DPPH values and the TFC ($r = 0.996$), as well as DPPH values and the TPC ($r = 0.866$), indicating that the phenolic compounds and mainly flavonoids may be major contributors to the free radical-scavenging function of "bili-bili" beer.

An inverse relationship was found between total bacteria and the alcohol content ($r = -0.967$), confirming the antibacterial effect of the alcohol [77]. The total fungi count was

Table 6: Mean scores of sensory attributes of "bili-bili" and "cochette."

Sensory attributes	Sampling sites (bili-bili)				
	Palar ($n = 10$)	Domayo ($n = 10$)	Ouro-tchédé ($n = 10$)	Pont-vert ($n = 10$)	p values*
Bitterness	$5.3 \pm 1.7^{a,b}$	4.7 ± 1.1^{a}	6.9 ± 1.5^{b}	7.0 ± 1.8^{b}	0.014
Alcoholic taste	6.1 ± 1.9^{a}	5.4 ± 1.2^{a}	6.5 ± 1.9^{a}	6.0 ± 1.7^{a}	0.578
Texture/viscosity	$6.4 \pm 0.9^{a,b}$	4.6 ± 0.7^{a}	7.3 ± 1.9^{b}	$6.1 \pm 1.5^{a,b}$	0.011
Flavor/odor	7.4 ± 1.7^{a}	6.1 ± 1.6^{a}	6.3 ± 1.7^{a}	5.8 ± 2.0^{a}	0.238
Color	7.5 ± 2.2^{a}	6.7 ± 1.2^{a}	6.8 ± 1.8^{a}	6.5 ± 2.3^{a}	0.690
Overall acceptability	$6.6 \pm 1.2^{a,b}$	5.6 ± 1.1^{a}	7.5 ± 1.1^{b}	$6.3 \pm 1.3^{a,b}$	0.014
	Sampling sites (cochette)				
	Palar 1 ($n = 12$)	Palar 2 ($n = 12$)	Pitoare ($n = 16$)	p values*	
Bitterness	5.2 ± 2.2^{a}	4.5 ± 2.2^{a}	6.3 ± 2.6^{a}	0.258	
Alcoholic taste	6.3 ± 2.3^{a}	5.5 ± 1.9^{a}	5.6 ± 1.6^{a}	0.629	
Texture/viscosity	6.0 ± 1.2^{a}	5.6 ± 1.5^{a}	6.4 ± 1.7^{a}	0.511	
Flavor/odor	6.9 ± 1.4^{a}	6.7 ± 2.6^{a}	7.0 ± 1.5^{a}	0.740	
Color	7.1 ± 1.2^{a}	7.2 ± 1.7^{a}	6.3 ± 2.3^{a}	0.493	
Overall acceptability	6.7 ± 1.3^{a}	6.8 ± 0.6^{a}	6.9 ± 1.5^{a}	0.435	

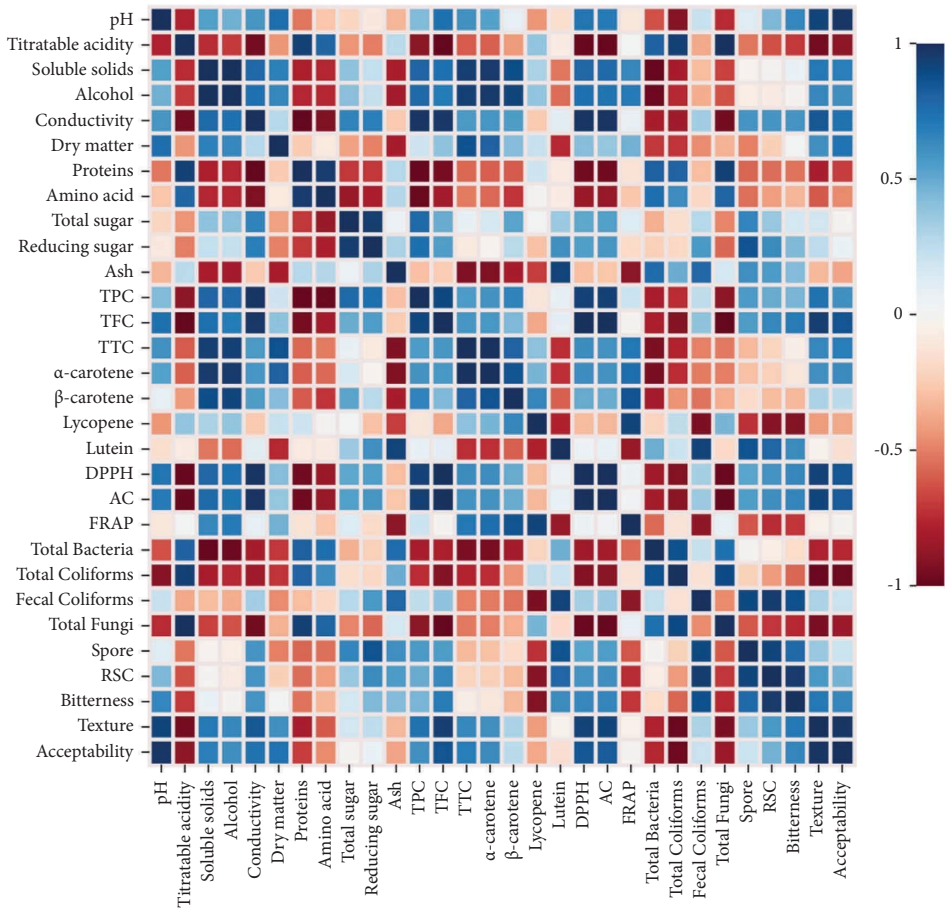
Two or more mean sensory scores with the same lower case superscript letters are not significantly different ($p > 0.05$) in the same row. p values* lower than 0.05 indicate significant differences in mean values of samples among the different locations.

Table 7: Safety scores of "bili-bili" and "cochette" samples sold in various sites of the city of Maroua.

Microbial scores*	Sampling sites (bili-bili)				Quality scale (cfu/mL)			
	Palar	Domayo	Ouro-tchédé	Pont-vert	m	3 m	M	S
Total count	46	46	46	46	10^6	3.10^6	10^7	10^9
Total coliforms	46	46	46	46	10^3	3.10^3	10^4	10^6
Fecal coliforms	0	0	0.4	1.5	10^2	3.10^2	10^3	10^5
Total fungi	46	46	46	46	10^5	3.10^5	10^7	10^8
Total mesophilic spore-forming bacteria	1.5	1.5	1.5	1.5	10^4	3.10^4	10^5	10^7
Fecal *Streptococcus*	0	0	0	0	10^3	3.10^3	10^4	10^6
Sulphite-reducing *Clostridia*	0	0	0	0	10^5	3.10^5	10^7	10^8
Salmonella and *Shigella*	0	0	0	0	0	0	0	0
Staphylococci spp.	0	0	0	0	10^2	3.10^2	10^3	10^5
Safety site scores	139.5	139.5	139.9	141				
Overall safety score	559.9							
Microbial score*	Sampling sites (cochette)				Quality scale (cfu/mL)			
	Palar 1	Palar 2	Pitoare		m	3 m	M	S
Total count	46	46	46		10^6	3.10^6	10^7	10^9
Total coliforms	46	46	46		10^3	3.10^3	10^4	10^6
Fecal coliforms	1.5	1.5	1.5		10^2	3.10^2	10^3	10^5
Total fungi	1.5	1.5	1.5		10^5	3.10^5	10^7	10^8
Total mesophilic spore-forming bacteria	1.5	46	1.5		10^4	3.10^4	10^5	10^7
Fecal *Streptococcus*	0	0	0		10^3	3.10^3	10^4	10^6
Sulphite-reducing *Clostridia*	0	0	0		10^5	3.10^5	10^7	10^8
Salmonella and *Shigella*	0	0	0		0	0	0	0
Staphylococci spp.	0	0	0		10^2	3.10^2	10^3	10^5
Safety site scores	96.5	141	96.5					
Overall safety score	334							

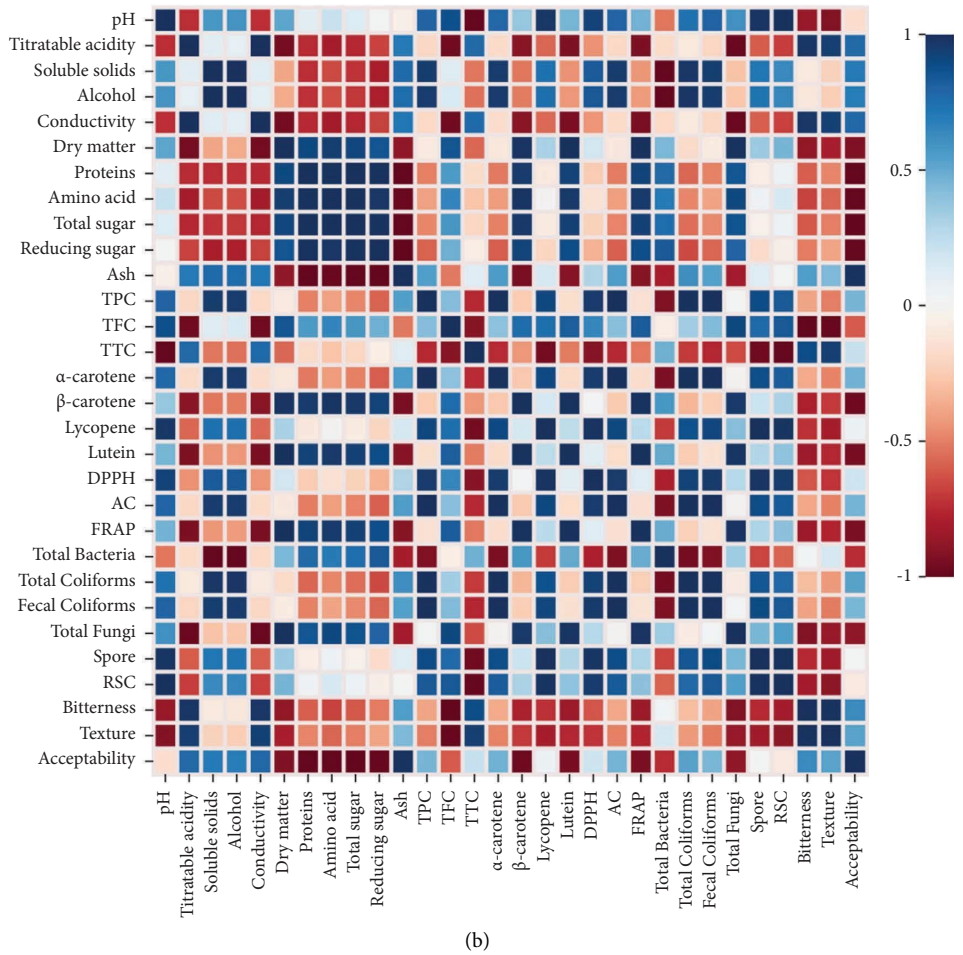
*The microbial score is based on the microbial count of each sample. It is assigned by comparing the microbial count to the referenced value (m) and the other m-based values; m: microbial reference value of each microbial group; $M = 10$ m (agar culture media); $S = 10^3$ m.

positively correlated to the titratable acidity ($r = 0.992$). This could be explained by their acido-tolerant character. Indeed, acidic environments typically stimulate fungi development. However, the TFC was negatively correlated to the fungi count ($r = -0.995$). This shows that flavonoids in "bili-bili" exert an antifungal activity [78]. There was a positive and significant connection between sulphite-reducing bacteria (SRC) and fecal coliforms ($r = 0.963$). This is not surprising given that both bacteria groups are typically associated with fecal contamination. The overall acceptability and total coliforms were shown to be negatively related ($r = -0.968$), implying that the most accepted "bili-bili" samples were the least contaminated with coliforms and thus the safest. This is highly interesting and could explain why "bili-bili" consumption is rarely associated with most cases of food-borne diseases.



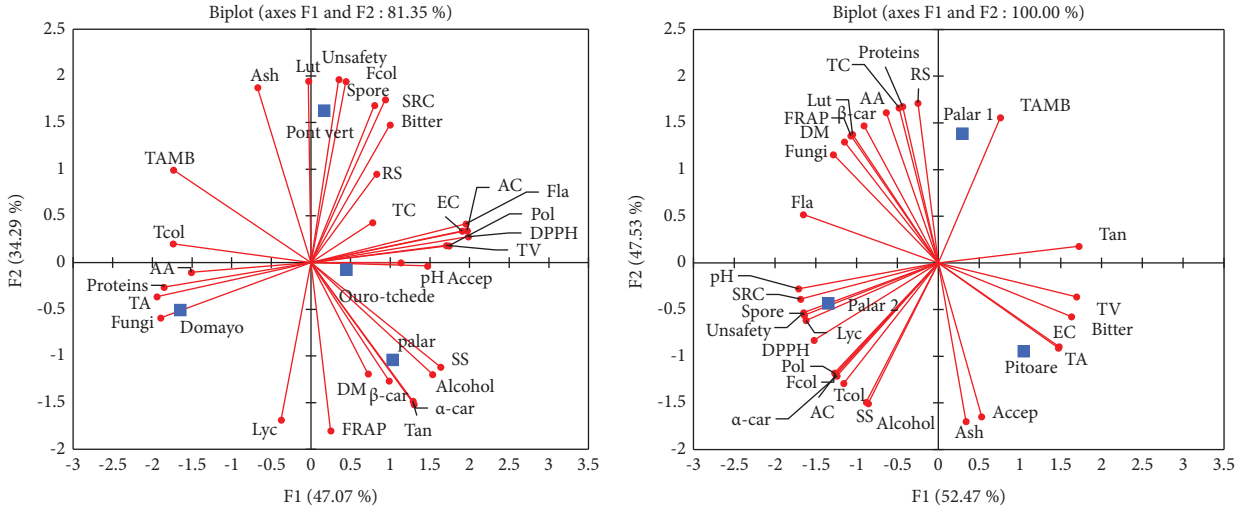
(a)

Figure 3: Continued.



(b)

Figure 3: Heat map Pearson correlation matrix of the physicochemical, proximate, sensory, microbiological properties, phenolic compounds, and antioxidant activities of "bili-bili" (a) and "cochette" (b). DPPH: DPPH scavenging activity; FRAP: ferric reducing antioxidant power; RSC: sulphite-reducing *Clostridia*; TPC: total phenolic content; TFC: total flavonoid content; TTC: total tannin content.



(a) (b)

Figure 4: PCA biplot of factor loadings and scores of "bili-bili" (a) and "cochette" (b) samples. AA: amino acid; α-car: α-carotene; AC: antioxidant capacity; Accep: acceptability; DM: dry matter; EC: electrical conductivity; Fcol: fecal coliforms; Fla: flavonoids; FRAP: ferric reducing antioxidant power; Lut: lutein; Lyc: lycopene; RS: reducing sugar; SRC: sulphite-reducing *Clostridia*; SSs: soluble solids; TA: total titratable acidity; TAMB: total aerobic mesophilic bacteria; Tan: tannins; TC: total carbohydrate; Tcol: total coliforms; β-caro: β-carotene; Pol: polyphenols.

The overall acceptability is positively correlated to pH ($r = 0.976$) and texture ($r = 0.973$). Bayoï et al. [14] found a comparable association between the overall acceptability of "téa lémi" and its pH ($r = 0.990$), and Bayoï et al. [15] reported a positive correlation between the overall acceptability of "kounou" and its texture ($r = 0.736$).

With regards to "cochette," a negative correlation was recorded between tannins and pH ($r = -0.998$). This correlation has a relevant nutritional interest because it indicates that the "cochette" samples with acidic pH levels had the highest tannin contents, and ingestion of such beverages should be avoided because of their limited nutritive value (presence of tannins) and health problems (stomach disorders and bone and teeth erosion) associated.

The FRAP value and lutein carotenoid equivalent were perfectly and positively correlated ($r = 1.000$) in "cochette," suggesting that lutein could potentially contribute to the ability to reduce Fe^{3+}. The TPC and α-carotene ($r = 1.000$), fecal coliforms and the TPC ($r = 1.000$), and fecal coliforms and α-carotene ($r = 1.000$) had the same connection. Furthermore, total coliforms and α-carotene were positively correlated ($r = 0.998$), as well as aerobic spore-forming bacteria and lycopene ($r = 0.999$). Both prior correlations show that the "cochette" samples with the poorest microbiological quality had the highest levels of polyphenols and carotenoids, both known as antimicrobials [79]. This could be explained by the fact that both groups of bioactive compounds had insufficient concentrations to provide an effective antimicrobial action.

The overall acceptability was negatively correlated to the proximate composition, including sugar ($r = -1.000$), proteins ($r = -0.998$), and free amino acids ($r = -0.998$). This means that the more the "cochette" was nutritive, the fewer the "cochette" was accepted. This is not surprising given that this beer, as most traditional beverages, is ingested with little knowledge of its intrinsic qualities (microbial, physicochemical, nutritional, etc.). In general, the consumer's choice and purchase decision are solely based on the sensory characteristics of the product [80].

3.8. Multivariate Analysis. In order to illustrate how the measured variables were loaded to the factors and the samples were distributed according to "bili-bili" and "cochette" characteristics, principal component analysis (PCA) was applied to the mean values of physicochemical, microbial, and sensory variables, and the obtained biplots are depicted in Figure 4. The first two factors of each biplot ($F1$ and $F2$) recorded eigenvalues above 1. Both factors explained 81.35% and 100% of the total variability for "bili-bili" and "cochette" samples, respectively. Considering "bili-bili," the first factor ($F1$) accounting for 47.07% was more associated with pH, total titratable acidity (TA), EC, protein and amino acid contents, TPC, TFC, scavenging activity (DPPH), aerobic mesophilic bacteria (TAMB), total coliforms (Tcol), fungi (TF), texture, and overall acceptability. The second factor (F2) explaining 34.29% of the total variance was mainly loaded with dry matter (DM) and ash contents, α-carotene (α-car), β-carotene (β-car), lycopene (lyc), and lutein (lut) carotenoid equivalents, tannin content (TTC), FRAP, fecal coliforms (Fcol), spore-forming bacteria (SFB), sulphite-reducing bacteria (SRC), and safety status.

According to PCA (Figure 4(a)), the "bili-bili" samples from Pont-vert related to the positive side of F2 were characterized by the bitterness, the largest ash and lutein levels, and the lowest safety level. This safety status appears to be explained by the highest microbial indicators such as Fcol, SFB, and SRC counts. Mainly connected to the left-hand side of $F1$, the samples from Domayo were essentially characterized by the highest TA, protein and amino acid contents, and TF count. The "bili-bili" samples from Palar, which are found in the space formed by the right hand of $F1$ and the side down of $F2$, were mainly associated with the highest levels of DM, alcohol, soluble solids (SSs), tannins, and α-car and β-carotene carotenoid equivalents. The samples from Ouro-tchédé were discovered in the same location as those from Palar, but they were too close to the origin and hence poorly represented. Indeed, Ouro-tchédé samples are associated with the third factor ($F3$) (data not shown), which was not taken into account for the biplot conception.

About PCA of "cochette" (Figure 4(b)), $F1$ and $F2$ accounted for 52.47% and 47.53% of the total variance, respectively. $F1$ was associated with pH, TA, EC, TFC, TTC, lyc equivalent, DPPH, texture, SFB, SRC, and safety, whereas $F2$ was related to alcohol, SS, proteins, amino acid (AA), total sugar (TC), reducing sugar (RS), ash, β-car equivalent, TAMB count, and overall acceptability. The samples from Palar 1 location, associated with the side up of $F2$, were defined by TAMB, RS, proteins, TC, AA, β-car and lyc equivalents, FRAP, and DM. "Cochette" sampled from the Palar 2 site was placed in the area formed by the $F1$ left-hand side and $F2$ side down. They were characterized by the pH, SRC, SFB, Fcol, and Tcol counts and the poorest safety status. The samples from Pitoaré found in the space defined by the positive side of $F1$ and the negative part of $F2$ were mainly characterized by the sensory attributes scores and the ash content.

4. Conclusion

To valorise "bili-bili" and "cochette," two fermented cereal-based beverages, the samples collected from various areas of Maroua were investigated. So, this study emphasized on their preparation procedure, as well as physicochemical features, quality attributes, and safety status of both local beers. Traditional "bili-bili" and "cochette" are made from sorghum and rice, respectively. Significant changes ($p < 0.05$) in physicochemical and microbial properties were observed, while most sensory aspects of both beverages showed no significant variation ($p > 0.05$). Both opaque beers were nutritionally rich, a good source of natural phenolic and carotenoid compounds with potential health benefits according to the scavenging activity and reducing power highlighted in vitro. However, these beers were highly contaminated with several microbial groups, suggesting poor handling practices and postproduction contamination given the acidic character of beverages. This calls for

awareness among the regular consumers in order to prevent food poisoning and stomach ulcer which may occur after repeated intake of these traditional acidic beers. Data generated in the current study provide crucial inputs to beer producers, vendors, and consumers. Furthermore, they could help regulatory authorities and policymakers in developing holistic preventive measures to protect against the consumption of unsafe beer. Therefore, there is a need to standardise these traditional beers by adhering to sanitary procedures and conducting routine quality control at every stage of the production, including raw materials, processing intermediates, and ready-to-serve drink.

Abbreviations

DM: Dry matter
GAE: Gallic acid equivalent
QE: Quercetin equivalent
TE: Trolox equivalent
TFC: Total flavonoid content
TPC: Total phenolic content
TSSs: Total soluble solids
TTC: Total tannin content
RACI: Relative antioxidant capacity index
PAC: Phenolic antioxidant capacity
TAMB: Total aerobic mesophilic bacteria
TCs: Total coliforms
FCs: Fecal coliforms
FS: Fecal *Streptococci*
TAMSFB: Total aerobic and mesophilic spore-forming bacteria
SRC: Sulphite-reducing *Clostridia*
YM: Yeasts and moulds.

Data Availability

The data used to support the findings of this study are included within this article.

Ethical Approval

The sensory study involving human subjects was approved by all the participants who gave their consent before the analysis. This part of the work was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Consent

All the participants gave their consent before the analysis.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

JRB was responsible for study conception, data analysis, paper drafting, and article revision. MBD was responsible for the procurement of samples, results analysis, and paper drafting. All the authors have read and approved the final version of the current manuscript and agreed to be accountable for all aspects of the work.

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